RECOMMENDED GUIDELINES FOR SAFE LEVELS OF SELENIUM AND MOLYBDENUM IN LIVESTOCK DRINKING WATER

A Report Prepared by

Ivan S. Palmer and Oscar E. Olson
Olson Biochemistry Laboratory
Chemistry Department
South Dakota State University
Brookings, SD 57007

Gerald M. Ward
Professor of Animal Science
Colorado State University
Fort Collins, CO 80523

Prepared for
California Regional Water Quality Control Board
Central Valley Region

March 1989

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FOREWORD

Nine Regional Water Quality Control Boards are responsible for regulating water quality in California. The Central Valley Regional Board (Region 5) is responsible for water quality regulation in the Sacramento and San Joaquin Valleys. These two watersheds cover 60 percent of the State and contain the nation's most productive irrigated agricultural lands. The success of this agricultural area has been the availability of plentiful good quality water supplies. These supplies are becoming increasingly limited and as the intensity of development has increased, these supplies have also experienced quality degradation. In some areas, particularly within the San Joaquin Valley, elevated levels of trace elements such as selenium and molybdenum have been detected as a result of return flows from irrigated agriculture.

As part of the regulatory process, the Regional Board must set water quality objectives for the protection of surface and ground water bodies for their designated uses. One of the designated beneficial uses for most waters in both the Sacramento and San Joaquin River Basins is for animal drinking water supply. Preliminary guidelines for animal drinking water were suggested by the National Academy of Sciences in 1972 for nationwide application. These guidelines were based on data developed prior to 1970. The data base available on trace elements and their effects, especially at low levels, was limited. Because of this limited data base, no guideline value could be established for molybdenum. The selenium guideline was established on the basis that most natural waters contained less than 50 micrograms per litre of selenium and no ill effects had been noted; therefore, this would be the interim level until further data could be developed to establish a more definitive number. In addition to the limited data on the specific trace element, no review of the interactions with other trace elements and salts was conducted. As a result of this limited data base and basis upon which the guidelines were established, using this data in its present outdated form as a basis for establishment of water quality objectives leaves considerable doubt about the protection of beneficial uses of water in the Central Valley.

This document reviews the prior data base as well as more current data for both selenium and molybdenum and recommends updated guidelines for animal drinking water. The review has been divided into three chapters. The first chapter contains a general overview of water consumption rates for a variety of livestock under various conditions. This overview sets the stage for the more detailed review of both trace elements. The second chapter discusses recent information on selenium nutrition and recommends guidelines for livestock water protection. Both

chapters were prepared by Professors Ivan S. Palmer and Oscar E. Olson of South Dakota State University and refer to a combined bibliography at the end of chapter two.

The final chapter, Chapter 3, was prepared by Professor Gerald M. Ward of Colorado State University. Chapter 3 discusses current molybdenum research and recommends guidelines for animal drinking water protection.

Both recommended guidelines attempt to identify factors in the San Joaquin Valley of California which would influence the recommendations. These factors include but are not limited to the arid to semi-arid environment, general poor quality water, including water high in sulfate, and extensive feedlot operations combined with large tracts of range land. Interactions with other elements are also reviewed, and recommendation for future studies have been included at the ends of Chapter 2 and Chapter 3 for the respective elements discussed.

CHAPTER 1: LIVESTOCK WATER CONSUMPTION

Ivan S. Palmer and Oscar E. Olson

Olson Biochemistry Laboratory
Chemistry Department
South Dakota State University
Brookings, SD 57007

A factor of considerable importance in recommending a safe concentration of any trace element in livestock waters is the intake (or consumption). It should be pointed out that intake is not the same as the water requirement of an animal. If water of good quality is supplied in unlimited amounts, its intake will usually exceed the requirement. Poor quality or restricted amounts of water may limit intake to less than that required for the good health of the animal.

Factors other than intake that must be considered in setting a standard for an acceptable livestock water for any of its constituents include:

1. Metabolic water formation. As much as 25 kg of water can be formed from 50 kg of feed within the animal body, and this can reduce water intake. (Morrison 1957, pp 163-4; Squires 1988, p 222)

2. Salinity of the water. As the salt concentration increases, water consumption often increases until a concentration that is toxic is approached, and then the consumption decreases. (Embry et al. 1959; NAS-EPA 1972, p. 306)

3. Water content of the feed. Succulent feeds can supply a significant portion of the total water intake of an animal. (Squires, 1988, pp 221-2; Merck 1951, pp 681 and 695; FWPCA 1968, p. 130).

4. Lactation. Particularly in dairy cows, a significant increase in the water requirement and thus in the water intake is noted during lactation (NAS-EPA 1972, p.305). However, much of the additional solutes derived from the increase in water intake should usually be excreted in the milk, so it is not very likely to be involved in any toxicity.

5. Animal characteristics. Several animal characteristics have some influence on the water intake of animals, including size, haircoat, activity, breed, age, and species (NAS-EPA 1972, pp. 304-5).

6. Habit. Habit may cause intake well beyond what is required for good health (Squires 1988, p 223).

The variety of factors influencing water intake complicates recommending a concentration for selenium or molybdenum in livestock drinking waters that will be safe but not excessively restrictive. The information in the following table speaks to this matter.

Table 1-1 Some estimates of the water intake of livestock and poultry

Animal	Comments	Water intake*	Reference
Beef cattle	Adult	26-45	FWPCA 1968, p130
	Nursing cow, 450 kg	60	NAS-NRC 1974,p31
	Finishing steer, 450kg	60	" " " "
	Steer, 2-yr old on dry feed	23-30	Merck 1961, p695
	Breeding cow on dry feed	38	" " "
	Fattening calf on dry feed	25-30	" " "
	Range cows (winter)	10	Morrison 1957, p708
	Range cows (summer)	44	" " "
Dairy cattle	Adult	38-61	FWPCA 1968, p130
	Lactating adult	90	NAS-NRC 1974, p31
	Growing heifer	60	" " " "
	Adult (maintenance)	60	" " " "
	Dry or lactating herd	57	Merck 1961, p681
	Cows yielding 36 L of milk/day may drink	136	Merck 1961, p681
Horses	Adult	30-45	FWPCA 1968,p130
	Medium work load, wt. 450 kg	40	NAS-NRC 1974, p31
	Lactating, wt. 450 kg	50	" " " "
	Average, mature	45	Merck 1961, p760
	Adult, hard work (alfalfa hay increases)	38-45	Morrison 1957, p827
Sheep	Ewes (winter)	3.8	Merck 1961, p707
	Nursing ewes	5.7	" " "
	Fattening lambs	1.9	" " "
	Sheep and goats	4-15	FWPCA 1968, p304

Table 1-1 Continued

Animal	Comments		Water intake*	Reference
Swine	Growing			
	23 kg	Fall	2.1	Merck 1961, p737
		Spring	2.6	" " "
	45 kg	Fall	3.6	" " "
		Spring	4.2	" " "
	68 kg	Fall	4.2	" " "
		Spring	4.7	" " "
	91 kg	Fall	4.5	" " "
		Spring	4.2	" " "
	136 kg	Fall	2.8	" " "
		Spring	2.8	" " "
	Adult		11-19	FWPCA 1968, p130
	Growing, 30 kg		6	NAS-NRC 1974, p31
	Fattening, 60-100 kg		8	" " " "
	Lactating sows, 200-250kg		14	" " " "
Poultry	Adult chickens		0.3-0.38	FWPCA 1968, p130
	Hens		0.14-0.18	Ewing 1963, p53
	Turkeys		0.38-0.57	FWPCA 1968, p130
	Turkeys	1-3 wk	0.042-0.095	Ewing 1963, p1109
		4-7 wk	0.14-0.32	" " "
		9-13 wk	0.35-0.54	" " "
		15-19 wk	0.63	" " "
		21-26 wk	0.51-0.64	" " "
	Chicken, 8-wk old		0.2	NAS-NRC 1974, p31
	Laying hen, 60% production		0.2	" " " "

* Liters per animal per day.

Winchester and Morris (1956) reported a very thorough study of the water intake of cattle under a variety of conditions, and some of their data were used in calculating the values for Table 1-2. These values reflect "total water intake" (water drank plus water contained in the feed) rather than "water consumption" ("free water"drank) or "water requirement" (equivalent of water from all sources, including metabolic, required for good health). The data are summarized in some detail since they provide information concerning the effects of so many factors.

Winchester and Morris (1956) have also shown the water intake of lactating dairy cattle to increase as the milk production increases.

Table 1-2 Total water intake of cattle under various conditions (Winchester and Morris 1956)

Animals	Weight	Ambient Temperature (degrees C)					
	(kg)	4	10	16	21	27	32
		(Water intake in liters/animal/day)					
Dairy cattle							
Heifers	91	7.6	8.3	9.5	11.0	12.5	18.2
"	181	14	15	17	20	23	33
"	272	19	20	24	28	32	45
"	363	24	26	30	35	40	57
"	454	28	30	34	41	47	66
"	545	30	33	38	44	51	72
Bulls	545	23	25	28	33	38	54
Lactating cows	530	64	76	80	83	79	72
Beef cattle (maintenance)	545	17	18	20	25	28	39
Wintering	363	23	25	28	33	---	---
Heifers & steers	363	24	26	30	35	40	57
Bulls	363	22	23	27	31	36	51
"	545	28	30	34	41	47	66
Fattening yearlings	363	25	27	31	37	42	60
Fattening 2-yr-olds	363	30	33	38	44	51	72

The information in Table 1-2 shows that temperature strongly effects water intakes and a temperature rise from 4°C to 32°C results in a 2.4-fold increase in of water intake. Obviously, any estimation of selenium or molybdenum intake based on water consumption would need to take into account ambient temperature.

More recently, Ray (1989) has conducted a study on the interrelationships of water quality, climate and diet on performance of feedlot steers over a two-year period in a hot arid climate (Arizona). In this study, the average summer maximum and minimum temperatures were 35.4°C and 17.5°C, respectively. The average winter maximum and minimum temperatures were 25.0°C and 8.0°C, respectively. Under these practical conditions, the average water intake for summer was 32.1 L/day and average feed intake was 6.25 kg/day (ratio of 5.1:1). For the winter months, the average water intake was 27.9 L/day and feed intake was 6.68 kg/day (ration of 4.1:1). These data indicate a less extreme effect of temperature on water intake than do the data of Winchester and Morris (1956) shown in Table 1-2.

CHAPTER 2: RECOMMENDED GUIDELINE FOR SAFE LEVELS OF SELENIUM IN LIVESTOCK WATERS OF THE SAN JOAQUIN VALLEY OF CALIFORNIA

Ivan S. Palmer and Oscar E. Olson

Olson Biochemistry Laboratory
Chemistry Department
South Dakota State University
Brookings, SD 57007

INTRODUCTION

In 1972, the National Academy of Sciences established guidelines for safe levels of selenium in drinking water for the U.S. Environmental Protection Agency. The recommended safe level for selenium in livestock drinking water was set at 0.05 mg/L. In accordance with contract No. 7-177-150-0 of the State of California Water Resources Control Board, this report will review the basis for the present guidelines and evaluate them in light of the more recent data.

Throughout this report, the term "livestock" will include beef and dairy cattle, sheep, swine, poultry, and horses. In some cases, other animals such as goats and mules may be included.

Types of Selenosis and Losses Expected

Excessive selenium intake can result in different types of poisoning that will be discussed later in this document. The type the animal suffers will depend upon the rate, amount, and, possibly, the chemical form of the element administered. One type is an acute toxicity caused by a large dose administered or consumed over a short period of time and causing clinical signs of toxicity, often including death (Rosenfeld and Beath 1964, pp.142-5). Chronic selenosis results from the ingestion or administration of sublethal amounts of the element over a period of time. One type of chronic selenosis is referred to as "alkali disease", since it was once thought to be caused by the consumption of highly alkaline water. It is now known to be caused by the ingestion of feeds and waters together containing less than a subacutely toxic concentration of the element over a period of a few weeks to months (Moxon 1937). Another type of what has been presumed to be chronic selenosis has been described as resulting from the ingestion of less than acutely toxic doses of selenium accumulator or indicator plants over a short period of time causing clinical signs that led to its having been called "blind staggers" (Rosenfeld and Beath 1964, pp.146-9). Van Kampen and James (1978) suggest, however, that in view of some of their studies the toxic principle in these plants is similar to

the unidentified toxic principle in loco weeds rather than being selenium related.

The concentration of selenium in waters is seldom, if ever, high enough to cause acute toxicity in livestock. Further, even were the "blind staggers" syndrome selenium-related, waters would very likely not be its cause because, from what we now know about the malady, an organic form of the element would probably be required at concentrations much higher than would be found in waters. Thus, major emphasis will be placed on the so-called "alkali disease" form of the poisoning in arriving at a recommendation for the maximum permissible concentration for the element in livestock water.

While deaths do occur among animals chronically poisoned by selenium, it is the other effects that cause the greatest losses in afflicted animals. These losses include, but are not restricted to, such things as loss of appetite and therefore failure to gain, sore hoofs making it difficult for the animal to take care of itself, failure of reproductive capacity, and occasionally a type of paralysis making competition with other stock for feed and water difficult.

Chemical Forms of Selenium in Natural Waters

Natural waters contain various chemical forms of the element, and these may have different toxicities. Some organic forms leach from seleniferous plants and reach streams or ponds in runoff, but their concentration is, except in very unusual circumstances, low. Sediments often contain inorganic forms, but some of these are tightly bound to the sediments or incorporated into them as a part of the mineral matrix and they have a low order of biological availability (Rosenfeld and Beath 1964, p. 41-55). Thus, they may contribute very little to the problem of toxicity. Elemental selenium may also be found in sediments, and this has usually been considered so highly insoluble as to be almost entirely unavailable to plants or animals. Recent studies, however (Echevarria et al. 1988), suggest that it may contribute much more to metabolizable selenium intake than usually suspected.

Generally speaking, in waters containing high concentrations of the element, the predominant chemical form is Se VI (selenate) (Stack et al. 1978). However, ponds or ditches in which algae or other plants grow contain much of their selenium in organic form (soluble or insoluble), much of which might be biologically available to animals. Se IV (selenite) is another inorganic form commonly found in waters, and it is the form that binds tightly to soil colloids. This keeps its concentration in most waters at a rather low level.

The various forms of the element may have somewhat different toxicities. From the standpoint of livestock drinking waters, however, an analysis for total soluble selenium should be the most reliable measure of the potential of a water to cause a selenium problem. Further, since the predominant form of the element in natural waters of high selenium content is the selenate (Stach et al. 1978), this is probably the form of choice for studies on the safety of livestock waters. Free selenite or selenate in waters would have somewhat similar toxicities (Schroeder 1967; Halverson et al.1962; Palmer and Olson 1974).

Interactions

A number of substances have been found to either increase or decrease the toxicity of selenium. Perhaps the first to be reported was the reduction of selenium toxicity by increasing the protein content of the diet (Moxon 1937; Smith 1939). About the same time, Moxon (1938) reported the protection of rats by arsenic against selenium in toxic diets. Later, it was found (Obermeyer et al. 1971) that methylated forms of the element, which had relatively low toxicities, became much more toxic on arsenic administration. This, of course, complicates the arsenic-selenium relationship, making care essential in evaluating arsenic as a preventive for selenosis.

Parizek et al. (1976) reported that pretreatment of rats with a single dose of selenite protected against dimethylselenide toxicity. Later, Kalauskova and Pavlik (1982) found that previous subcutaneous injections of selenite, selenate, selenomethionine, or dimethylselenide protected rats against selenite toxicity. Such an apparent adaptation had previously been suggested by other workers (Ermakov and Kovalski 1968; Jaffe and Mondragon 1975). The importance of the adaptation factor is not well established, but deserves some study in livestock.

Sulfate has long been known to greatly reduce the uptake of selenate by plants (Hurd-Karrer 1938). Recent reports on California studies (Mikkelsen et al. 1988; Albasel et al. 1989) have verified this finding. In work based on the early report, Halverson and Monty (1960) found that, when rat diets were supplemented with toxic concentrations of selenate, sulfate would reduce their toxicity although the practical significance of this observation, under field condition, is not clear. Sulfate did not, however, appreciably reduce the toxicity of diets supplemented with selenite (Halverson et al. 1962).

Of several protein sources studied, linseed oil meal added to diets containing toxic concentrations of selenium gave more protection than did several other proteins (Moxon 1941). More recently, the protective factors in the meal were found to be two closely related cyanogenic glycosides (Palmer et al. 1980), the cyanide released from them being the protective factor (Palmer

and Olson 1980). The occurrence of cyanide in any natural water in amounts high enough to be of significance in reducing selenosis without the cyanide, itself, being toxic seems remote.

Interesting interactions between selenium and mercury have been reported in the literature and reviewed by Parizek (1976). Selenite is highly potent in reducing mercury toxicity, and mercury can reduce selenium toxicity (Hill 1974). However, mercury does increase the toxicity of dimethyl selenide (Parizek et al. 1980). Chronic poisoning by methyl mercury can be reduced by increasing the selenium content of the food.

Other interactions with metals, as follows, have been reported: cadmium, thalium, and silver (Parizek 1976), lead (Flora et al. (1983), and copper (Jensen 1975). In addition to these, a number of organic compounds affect the toxicity of selenium. No effort is made here to review them. With the exception of the effect of protein in reducing selenium toxicity and the sulfate effect on selenate mentioned earlier, none of the selenium antagonists reported to date appear to offer a practical approach to resolving the toxicity problem. Neither has synergism been reported to be of significance in increasing the selenium toxicity under field conditions. It appears, therefore, that the consideration of interactions in establishing a standard for selenium in livestock drinking waters is a very complex matter and is impractical.

Toxicity of Selenium in Feed Versus Water

Only a few studies on the toxicity of selenium in water have been reported, and comparisons of this toxicity with that of selenium in feeds are not available. The data in Table 2-1 were gleaned from an unpublished study with rats at the South Dakota Agricultural Experiment Station. Sodium selenite was added to distilled water or to a diet (80.8% corn, 12% casein, 3% corn oil, 2.2% vitamin mix, 2% salts IV) to give 5 μg Se/g. Two groups of albino rats were fed the selenized diet with distilled water or the diet without added selenium and with distilled water with the added selenium. Feed and water intake, survival, and animal weight records were kept. The experiment was terminated at six weeks.

The experiment was run twice, with five rats per treatment and with selenium added to the feed of two groups each time. The data from the two experiments were combined and are presented in Table 2-1. In an earlier study with rats (Halverson et al. 1966) under very similar conditions, the ratio of water consumption to feed consumption was 1.73 ± 0.25, while in this study it was about 1.0. The reason for this difference is not obvious, but in this study the initial weight was about 10 g greater. There is

no clear evidence in Table 2-1 that the toxicity of the element was different for the two sources, feed or water.

Table 2-1 Comparison of performance of rats with selenium in feed versus selenium in water.

	Group 1 Se in feed (5 μg/g)	Group 2 Se in water (5 μg/mL)	Group 3 Se in feed (5 μg/g)
Average terminal weight of rats (g)	251	237	236
Number of survivors	8/10	6/10	6/10
Average Se intake μg/rat/week	442	432	452

REVIEW OF TOXICITY OF SELENIUM TO ANIMALS

Relative to establishing the tolerance of animals to selenium (Se) in the drinking water, it is appropriate to review the established levels of toxicity of the element. A number of reviews have been performed on this point (Amor and Pringle 1945; Burk 1976; Cerwenka and Cooper 1961; Cooper 1967; Cooper and Glover 1974; Fishbein 1977; Moxon 1937; Moxon and Rhian 1943; NAS-NRC 1976; NAS-NRC 1983; Olson 1978; Rosenfeld and Beath 1964; Shapiro 1973; WHO 1987; Wilber 1980). Many of these were published after the 1972 Guidelines were established. One of the most recent reviews involved recommendations for safe intake by humans (Olson 1986). The latter review suggests that the terms acute, subacute, and chronic selenosis, as suggested by Rosenfeld and Beath (1964), are most appropriate in describing the short, medium and long term effects of selenium.

With respect to drinking water standards, chronic toxicity is undoubtedly of greatest concern. However, acute and subacute toxicity data do provide information as to comparative toxicities, variation of toxicity among species, etc. Even though the main interest for this review is in domestic farm animals, data for other animals will be reviewed since most of the information on comparative toxicities have been compiled on laboratory animals.

Acute Toxicity

Data from a number of investigators have recently been summarized by Olson (1986), and Table 2-2 shows values for acute toxicity to mammals for those forms of selenium most likely to occur in water.

It should be noted that in order to provide the comparative data shown in Table 2-2, it was necessary to make some assumptions and calculations from the data in the original references. Furthermore, strict interpretations of the data are made difficult by reported variability in toxicity due to species (Smith et al. 1937), age (Ostadalova et al. 1978), sex (Parizek et al. 1980), route of administration (Muehlberger and Schrenck 1928; Ammar and Couri 1981), and previous exposure to selenium (Parizek et al. 1976a).

If one examines the data in Table 2-2 for a single species such as the rat, for which the same route of administration and criteria of toxicity are used, it appears the commonly occurring forms of selenium have very similar acute toxicities. The intraperitoneal 48 hour LD_{75} values for selenite, selenate, selenocystine and selenomethionine are 3.2-3.75 mg Se/kg bw (body weight), 5.25-5.75 mg Se/kg bw, 4.0 mg Se/kg bw and 4.25 mg Se/kg bw, respectively.

Table 2-2 Acute Toxicities of Various Forms of Selenium

Form of Se	Kind of Animal	Route*	Toxicity in terms of Selenium	Reference
Sodium selenite				
Na_2SeO_3	Rat	Oral	3.2 mg/kg bw (10day LD_{50})	Cummins and Kimura (1971)
			4.8-6.0 mg/kg bw (LD_{50})	Pletnikova (1970)
		I.V.	5.7 mg/kg bw (LD_{50})	Muehlberger and Schrenck (1928)
			3 mg/kg bw (min. lethal dose)	Smith et al. (1937)
		I.P.	3.2-3.75 (48-hr LD_{75})	Franke and Moxon (1936)
	Pony	Oral	6 mg/kg bw killed 2 of 4 animals	Stowe (1980)
	Horses & Mules	Oral	3.3 mg/kg bw (min. lethal dose)	Miller and Williams (1940)
	Pigs	Oral	13-18 mg/kg bw (min. lethal dose)	Miller and Williams (1940)
	Pigs (Se adequate)	I.M.	1.4 mg/kg bw (48-hr LD_{50})	Van Vleet et al. (1974)
	Pigs (Se deficient)	I.M.	0.9-1.0 mg/kg bw (48-hr LD_{50})	Van Vleet et al. (1974)
	Dog	I.V.	0.88-1.0 mg/kg bw (24-hr LD_{50})	Heinrich and Maccanon (1957)
		S.C.	1.5-2.0 mg/kg bw (24-hr LD_{50})	Anderson and Moxon (1942)
	Rabbit	Oral	3 mg/kg bw (min. lethal dose)	Smith et al. (1937)
			2.25 mg/kg bw (LD_{50})	Pletnikova (1970)
			2.85 mg/kg bw (LD_{50})	Muehlberger and Schrenck (1928)
			3.9 mg/kg bw (24 hour LD_{50} dose)	Berschneider et al. (1976)
		I.V.	1.5 mg/kg bw (min. lethal dose)	Smith et al. (1937)
			0.9 mg/kg bw (LD_{50})	Muehlberger and Schrenck (1928)
		S.C.	1.8 mg/kg bw (min. fatal dose)	Levine and Flaherty (1926)
	Mouse	Oral	3.2-3.5 mg/kg bw (LD_{50})	Pletnikova (1970)
		I.V.	2.3 mg/kg bw (24-hr LD_{50})	Ammar and Couri (1981)
		I.C.	0.14 mg/kg bw (24-hr LD_{50})	Ammar and Couri (1981)
	Calf	I.M.	1-2 mg/kg bw (lethal dose)	Macdonald et al. (1981)

Table 2-2 Continued

Form of Se	Kind of Animal	Route*	Toxicity in terms of Selenium	Reference
Na_2SeO_3	Cow	Oral	9.9-11.0 mg/kg bw (min. lethal dose)	Miller and Williams (1940)
	Guinea pig	Oral	2.3 mg/kg bw (LD_{50})	Pletnikova (1970)
	Cat	I.V.	2-3 mg/kg bw (min. lethal dose)	Smith et al. (1937)
	Sheep	I.M.	0.7 mg/kg bw (8 day LD_{50})	Blodgett and Berill (1987)
Sodium Selenate (Na_2SeO_4)	Rat	I.V.	4.3 mg/kg bw (LD_{50})	Muehlberger and Schrenck (1928)
		I.P.	5.25-5.75 mg/kg bw (48-hr LD_{75})	Franke and Moxon (1936)
	Rabbit	Oral	1.8 mg/kg bw (LD_{50})	Muehlberger and Schrenck (1928)
		I.V.	1.1 mg/kg bw (LD_{50})	Muehlberger and Schrenck (1928)
H_2Se	Guinea pig	Inhaled	All animals exposed to 0.47 mg/L of air for 10 min died by 5 days	Dudley and Miller (1937)
			All animals exposed to 0.02 mg/L of air for 60 min died by 25 days	Dudley and Miller (1937)
			0.001 mg/L of air (8-hr LD_{50})	Blodgett and Berill (1987)
Elemental Se	Rat	Oral	6700 mg/kg bw (10 day LD_{50})	Cummins and Kimura (1971)
		I.P.	over 1000 mg/kg bw for death	Hall et al. (1951)
Se-Cystine	Rat	I.P.	4.0 mg/kg bw (48-hr LD_{75})	Moxon (1940)

Table 2-2 Continued

Form of Se	Kind of Animal	Route	Toxicity in terms of Selenium	Reference
Se-Methionine				
	Rat	I.P.	4.25 mg/kg bw (48-hr LD_{75})	Klug et al (1949)
	Mouse	I.V.	8.9 mg/kg bw (24-hr LD_{50})	Ammar and Couri (1981)
		I.C.	5.3 mg/kg bw (24-hr LD_{50})	
$(CH_3)_2Se$	Rat	I.P.	1600 mg/kg bw (24-hr LD_{50})	McConnell and Portman (1952)
	Mouse	I.P.	1300 mg/kg bw (24-hr LD_{50})	McConnell and Portman (1952)
$(CH_3)_3SeCl$	Rat	I.P.	49.4 mg/kg bw (4-hr LD_{50})	Obermeyer et al. (1971)

* I.M. - Intramuscular
I.P. - Intraperitoneal
I.V. - Intravenous
S.C. - Subcutaneous
I.C. - Intracerebroventricular

The similarity in magnitude of toxicity of the commonly occurring selenium compounds is also supported by the acute toxicity data in chick embryos where such factors as differences in rate of absorption should be minimized. The data in Table 2-3 (taken from Olson 1986) show that selenite, selenate, selenomethionine and selenocystine have similar toxicities in embryos of the same age. Selenocystine is less toxic than the

Table 2-3 Toxicities of various forms of selenium injection into air sacs of fertile chicken eggs

	Embryo age at injection (days)	LD_{50} as mg Se/kg of egg contents
Sodium selenite	4	0.1[a]
	14	0.51[b]
Sodium selenate	4	0.13[a]
	14	1.76[b]
Selenocystine	4	0.64[a]
Selenomethionine	4	0.13[a]
Methylseleninic acid	4	0.052[a]
Se-Methylselenocysteine	4	0.57[a]
Dimethylselenoxide	4	6.53[a]
Trimethylselenonium chloride	4	15.7[a]

[a] From Palmer et al. (1973)
[b] From Halverson et al. (1965)

other substances in this system but there is no more than a 6-fold difference. Methylseleninic acid was the most toxic compound, but this substance has not been studied in other species. The methylated derivatives, proposed as end products of metabolism in other species, were relatively nontoxic in chick embryos.

It is not possible to unequivocally list an order of toxicity for the various selenium compounds of interest. It is usually possible to find exceptions to the order depending on the

species, route of administration or criteria of toxicity. In a comprehensive review of the literature, Olson (1986) suggested data such as that in Table 2-2 indicated the order of toxicity to be H_2Se > selenite > selenate > selenocystine > selenomethionine. In a recent review by the World Health Organization (WHO 1987), the same order of toxicity prevailed. Some studies reverse the order of selenocystine and selenomethionine (Palmer et al. 1973). The acute toxicities of inorganic and organic selenium are usually close in order of magnitude. Vinson and Bose (1987) have recently presented data that the oral LD_{50} of selenite and high-selenium yeast (a protein hydrolysate) were 12.7 mg Se/kg and 37.3 mg/kg, respectively. Methylated forms usually are less toxic than other forms with the exception of methylseleninic acid (see Tables 2-2 and 2-3).

The LD_{50} values for sodium selenite administered **orally** to various species have been determined by Pletnikova (1970) and are as follows: male white mouse (7.75 or 7.08); female albino rat (10.50, or 13.19); female guinea-pig (5.06); female rabbit (2.25). Where two values are given, it is the result of evaluating the data by different statistical procedures. These toxicities appear to be about one half of those in Table 2-2.

Smith et al. (1937) have reported that the minimum lethal dose as sodium selenite or selenate in rabbits, rats and cats was 1.5 - 3 mg/kg regardless of whether the substances were administered orally, subcutaneously, intraperitoneally or intravenously. This lack of effect of mode of administration probably reflects the rapid absorption of soluble selenium compounds from the site of injection or from the gastrointestinal tract (Glover et al. 1979). The apparent small effect of the mode of administration on the toxicity of selenium compounds is also supported by other data in Table 2-2, although direct comparisons are difficult to make. Although for the preceding reviewed data, it can not be stated unequivically that orally administered selenium is less toxic than parenterally administered selenium, there appear to be considerable data that support this assumption.

Several studies on acute selenium toxicity that appear in the literature do not permit calculation of LD_{50} values but they do provide useful estimates of the toxicity range. These studies have been reviewed by Olson (1986). As a part of the effort to prove selenium the cause of the alkali disease syndrome, Franke et al. (1936) injected sodium selenite or selenate into the air sacs of fertile chicken eggs. They found that 0.6 ppm Se (based on an assumed 50 g egg content weight) as selenite or 0.8 ppm Se as selenate caused increased death or abnormal development of the embryos, including shortened or malformed upper beak, underdeveloped body, missing eyes or legs, and an ectopic condition. These signs were similar to those reported by Sukra et al. (1976) who also reported gastroschisis. Khan and Gilani

(1980) reported similar findings along with several alterations characteristic of cell damage observed in electron microscopic examinations on injecting up 1.0 ppm Se as selenite into the air sac. Kury et al. (1967) injected up to 18 μg Se/egg (about 0.36 ppm) as selenite in the yolk at 4 days incubation and found it to cause lower red blood cell counts and hemoglobin levels at 19 days, along with some deformed embryos. A tendency for asymmetrical expression of certain of the embryonic malformations has been suggested (Landauer 1940). Ridgway and Karnofski (1952) reported that injecting 0.4 ppm Se as selenious acid into the yolk early in the incubation caused toxic effects.

Neethling et al. (1968) injected two adult wethers intravenously with 4 mg Se/kg bw as selenite and both died within 20 minutes. Two others, injected intravenously with 3.4 mg Se/kg bw as selenomethionine, died within 10 hours. Few signs of toxicity were observed except for cyanosis and pulmonary edema. The same authors suggested that toxic but sublethal doses of selenomethionine or selenocystine, repeated a few times at weekly or monthly intervals, had cumulative effects. Blodget and Berill (1987a) studied the pharmokinetics of intramuscularly administered sodium selenite. Four sheep each were injected with 0.4, 0.6, and 0.7, 0.8, 1.0 mg Se/kg bw. Only one animal in the 0.7 mg/kg group died in 192 hours. All animals in groups received 0.8 and 1.0 mg Se/kg died.

Orstadius (1960) has reported that subcutaneous injection of 1.2 or 2.0 mg Se/kg body weight as selenite caused death in pigs in 5 days and 4 hours, respectively. Doses of 0.9 to 1.1 mg Se/kg caused no signs of toxicity except a slight to moderate elevation in SGOT (serum glutamic oxaloacetic transaminase) level. Herigstad et al. (1973) found that administration of 3 mg Se/kg bw as selenite to two pigs caused death in 2 1/2 and 14 hours. Diehl et al. (1975) injected 18 kg pigs intramuscularly with selenite at levels of 0.0, 0.275, 0.55, 1.10, 1.65, 2.20, 2.75 and 3.3 mg Se/kg bw. Death occurred within 6 days in all animals receiving 1.65 mg Se/kg or greater.

Several recent field reports have linked elevated dietary selenium to a paralysis of the rear quarters of swine accompanied by histological lesions in the spinal cord referred to as porcine focal symmetrical poliomyelomalacia (PFSP), (Wilson et al. 1983; Harrison et al. 1983). On the basis of the levels of selenium observed in the accidental poisonings, Wilson et al. (1988) administered daily capsulated doses of sodium selenite to pigs at levels of 1.4 mg, 2.6 mg and 4.2 mg/kg bw (equivalent to 0.64, 1.19, and 1.91 mg Se/kg bw, respectively). Sodium selenite at 4.2 mg/kg was highly toxic since 2 of 4 pigs were dead or recumbent within 3 days and the other two were dead or euthanized between days 6 and 9. Sodium selenite at 2.6 mg/kg produced clinical signs of PFSP in all four treated pigs between 5 and 8

days of treatment. At the level of 1.4 mg/kg, only one pig developed signs of PFSP while 3 pigs remained clinically normal. Thus the maximum repeated oral dose allowed without production of clinical signs would be less than 0.6 mg Se/kg bw.

An accidental case of poisoning in calves due to a miscalculation has been reported by Shortridge et al. (1971). The average injected dosage for the animals was reported to be 0.5 mg Se/kg bw as selenite. Of 557 animals, 67% died over a five week period with some deaths occurring within two hours.

The accidental poisoning of 180 of 190 lambs by the ingestion of an estimated 6.4 mg Se/kg bw as selenite has been reported (Gabbedy and Dickson 1969). In addition, Lambourne and Mason (1969) have reported the death of 72 of 203 lambs in 48 hours after the administration by drench of an average of 1.7 mg Se/kg bw as sodium selenite. A dosage of about 0.6 mg Se/kg bw caused no untoward effects in 450 additional animals.

Ostadalova et al. (1978) has reported that a single subcutaneous dose of about 1.6 mg Se/kg bw as selenite will cause cataracts in male suckling rats. This has been confirmed by others (Bhuyan et al. 1981; Bunce and Hess 1981; Shearer et al. 1980). The period for cataract induction lies between 2 and about 17 days postnatal (Ostadalova and Babicky 1983). Selenate, DL-selenomethionine, and DL-selenocystine have a similar effect (Ostadalova and Babicky 1980). Oral exposure to selenium has also been reported to cause cataracts (Shearer et al. 1983).

The signs of acute toxicity have been recently reviewed by Olson (1986). Although they vary with the rate and amount of consumption, route of administration, and the chemical form of selenium, and the animal species involved, a summary of them would include: garlic odor of breath, dyspnea, pulmonary edema, tachycardia, emesis, diarrhea, depression, ataxia, incoordination, paralysis, anorexia, and excessive salivation. Analysis of the blood or urine is probably the most helpful test for diagnosing acute selenosis.

Chronic Toxicity

In 1937 Moxon (p. 12) stated that, generally, selenium poisoning of the alkali disease type results when animals consume feeds containing 5-40 ppm of the element over a period of time. A more recent extensive review concludes that normal diets containing 4 to 5 ppm Se will usually show inhibition of growth (NAS-NRC 1976). This evaluation of the borderline level for toxicity was corroborated by another review by Olson (1978).

A body of literature exists, however, that suggests levels of less than 5 ppm of the element in the diet may be toxic depending on the criteria used (NAS-NRC 1976). The review which follows

will attempt to suggest a "no effect" level for the element although the literature for the complete range of toxicity will be reviewed. This summary relies heavily on the compilation of literature by Olson (1986).

Laboratory animals-Munsell et al. (1936) found no effect of 1.5 ppm Se as seleniferous wheat on growth and reproduction in the rat. However, at 6 ppm Se, both growth and reproduction were below normal. Moxon (1937) reported that rats fed 4.38 ppm Se from corn showed a decrease in growth and an increased liver atrophy. Franke and Painter (1938) reported that less than 5 ppm Se in rat diets would reduce growth.

In a long term study, Smith et al. (1937) administered graded doses of selenite or selenate orally to rabbits in the range of from 0.3 to 1.5 mg Se/kg bw per day for a period of over three months. At the 0.3 mg level, six of 9 animals receiving selenite died and 3 of 5 animals given selenate died. When 0.3 mg Se/kg as selenite was given daily by intravenous injection, 8 of 10 died. In some rabbits a cumulative dose of 11 to 13 times the minimum lethal dose was tolerated. If one assumes an average body weight of 3.7 kg and an average food consumption of 90 g/day (2.5% of body weight), the daily administration of 0.3 mg Se/kg bw would be equivalent to receiving a diet containing 12 ppm Se.

Moxon and Rhian (1943) summarized several older studies that indicated diets containing 5 ppm Se/kg or more caused chronic selenosis in several species such as chickens, rats and dogs. Later Fitzhugh et al. (1944), reported that rats fed diets with selenium supplied by wheat or corn at graded levels between 3 and 20 ppm, showed signs of toxicosis as characterized by decreases in growth, food intake and the occurrence of some liver cirrhosis.

Smith and Lillie (1940) concluded from their studies on chronic selenosis in cats and rabbits that an intake of naturally occurring selenium of 1.0 mg Se/kg bw per day could be tolerated without causing serious symptoms or pronounced tissue damage. However, as little as 0.2 mg Se/kg bw per day might cause minor signs of systemic poisoning. If one assumes an average body weight of 3.3 kg for cats and 3.7 kg for rabbits and a respective feed intake of 2.5% of the body weight, the selenium intake of 0.2 mg Se/kg bw would be equivalent to consuming a diet containing 8.0 ppm Se.

Rosenfeld and Beath (1954) fed diets containing 1.5 or 2.5 ppm Se as selenate to rats through two generations without effect on reproduction other than a reduced number of offspring reared by the second generation with the 2.5 ppm diet. However, 7.5 ppm Se prevented reproduction in females without affecting the fertility of the males.

Halverson et al. (1966) fed graded levels of selenium as selenite or from wheat to post-weanling rats. With a slight growth reduction from the selenite diet at 4.8 ppm Se, growth retardation occurred at the 6.4 ppm Se level for both forms of the element. Increased spleen weight and altered liver appearance also occurred at this level. At 8.0 ppm Se, enlargement of the pancreas, reduction of blood hemoglobin content, and elevation of serum bilirubin content were observed. Later, Halverson (1974) fed rats selenite in a Torula yeast or a casein-based diet at graded levels and found growth depression, mortality, and enlargement of kidneys and spleen to result from 2.5 ppm Se in the casein diet and from 5.0 ppm Se in the yeast diet. Reproduction was not adversely affected by selenium in these experiments in which 5.0 ppm was the highest level fed.

It has been reported (Schroeder 1967; Schroeder and Mitchener 1971) that 2 ppm Se as selenite in the drinking water of rats is extremely toxic as compared to selenate. This concentration of selenite in the drinking water of mice was well tolerated. However, it should be noted that Schroeder and coworkers, have stated in other sources (Schroeder, 1968; Schroeder et al. 1970) that the concentration of selenite selenium in their studies was actually 3 ppm and not 2 ppm. This has been reiterated by Frost (1971). Others (Palmer and Olson 1974) have reported that 2 or 3 ppm Se as selenate or selenite in the drinking water of growing rats caused only a slight reduction in weight gains, 6 or 9 ppm being very toxic. The difference in toxicity of the two forms was not significant. Up to 2.8 ppm Se as selenite in the drinking water of gerbils had no effect on the growth rate of juveniles but slightly increased the weights of young adults (Lalor and Llewellyn 1979). Histopathological effects and increased blood iron retention suggested some toxic effect, being greater for selenite than for selenate. The addition of selenite at 3 ppm Se to the drinking water of mice caused mild signs of toxicosis at 50 days (Jacobs and Forst 1981).

McAdam and Levander (1987) fed rats torula yeast diets which contained 2.5,5.0 or 10.0 μg Se/g diet as D-selenomethionine, L-selenomethionine, sodium selenite or sodium selenate. All rats consuming 10 μg Se/g of diet, died within 29 days whereas those fed 2.5 μg/g showed no depressed growth and all survived except for 2 out of 8 receiving sodium selenate.

With regard to relative toxicities of various forms of selenium, Franke and Painter (1938) found the following order of toxicity: selenium from wheat was more toxic than that from corn, followed by that from barley, selenate and selenite. Others (Munsell et al. 1936) have found a similar order of toxicity on comparing the toxicities of selenious acid with grain

or when comparing selenite with L-selenocysteine (Moxon et al. 1941). Other work has suggested a different order of toxicity for inorganic and organic forms of selenium with selenite being the most toxic (Halverson et al. 1966; Halverson et al. 1962; Palmer et al. 1983; Smith and Lillie 1940). Direct comparison in toxicity of selenite, selenomethionine, seleniferous corn and Se from Brazil nut meal, when fed to rats, has shown that selenite was most toxic with seleniferous corn second (Palmer et al. 1983). Various studies (Halverson et al. 1962; Palmer et al. 1983; Goehring 1983) have indicated that when the rat is the experimental animal, equivalent levels of selenium are more toxic when fed in corn based diets than when fed in wheat based diets. Herr (1985) has provided data that suggest that the relative amino acid balance of the protein is adequate to explain the differences in toxicity of the selenium in wheat-based and corn-based diets. It should be noted that the magnitude of the toxicity differences between diets with different protein sources is usually quite small.

Farm animals-The signs of toxicity observed by early workers for chronic selenosis of the alkali disease type have been summarized by Moxon (1937), and Draize and Beath (1935). A more recent restatement of the signs has been furnished by Olson (1986) as follows:

Horses: Loss of long hair from the mane or tail, inflammation at the coronary band followed by cracked hoofs, rough hair coat, and emaciation.
Cattle: Inflammation at the coronary band as with horses and at the junction of the horns and hide, followed by cracking or malformation of the hooves or horns, loss of hair from the switch, erosion of the joints of the long bones, emaciation, atrophy of the heart, and irritation of the gastrointestinal tract.
Sheep: Kidney damage, but other wise few signs.
Swine: Hoof lesions similar to those for horses and cattle, loss of body hair and emaciation.
Poultry: Failure of eggs to hatch due to deformed embryos.

In addition to the above, failure of reproduction in cattle (Dinkel et al. 1963) and swine (Wahlstrom and Olson 1959), and focal symmetrical poliomyelomalacia in swine (Wilson et al. 1982; Wilson et al. 1983; Wilson et al. 1988) have been observed.

Instances of experimentally produced chronic selenosis in horses sheep and cattle are rare. Knott et al. (1958) fed an aged gelding Miranda reticula which contained adequate selenium to provide a total of 13.3 g Se in 82 days. This resulted in a loss of hair and lameness. The first sign of lameness occurred in 56 days after the animal had consumed 6 g of Se. Miller and Williams (1941) produced loosening of hair, inappetence, emaciation and hoof lesions in a horse and mule given the

equivalent of 24 and 17 ppm Se, respectively, sprinkled on oats or as a drench.

Sodium selenite was administered orally to 245 kg steers on a grain-hay-molasses diet by Maag et al.(1960). They found that the animals tolerated doses of 0.25 mg Se/kg body weight three times weekly (equivalent to 0.12 mg Se/kg body weight per day), but that doses of 0.5 mg Se/kg body weight given three times a week caused death or a variety of symptoms. Olson and Embry (1973) fed a diet containing 15 ppm Se as selenite to 5 heifers for 23 days. Four animals were unaffected but one exhibited decreased weight gain, sorefootedness, cracked hoofs and excitability.

Relative to selenium toxicity in sheep, Rosenfeld and Beath (1947) have reported gross malformation in the eyes of lambs from ewes on seleniferous range. Glenn et al. (1964) orally administered up to 50 mg Se/day as selenite to sheep weighing about 50 kg. No weight losses occurred in 72 days. However, several deaths occurred between 78 and 178 days in animals receiving 25 to 50 mg/day (0.5 to 1.0 mg Se/kg bw, respectively. Assuming a feed consumption of 2.5% body weight, this dosage would be equivalent to ingesting a diet containing 20 to 40 ppm Se, respectively. Few external signs of toxicity were noted other than death. The World Health Organization Task group on Selenium (WHO 1987) has reviewed the work of Ermakov and Kovalskij (1968) which described signs of chronic selenium toxicity under conditions where sheep were fed feeds containing 2 mg Se/kg feed (fresh weight). Signs observed were hoof deformation, loss of wool, hypochromic anaemia, increase in the activity of both alkaline and acid phosphatase.

Considerable data are available on chronic selenosis in poultry. Poley and Moxon (1938) fed 2.5 ppm Se from grain in the diet of laying hens and found no effect on hatchability. However, 5.0 ppm reduced it slightly and 10 ppm reduced it to zero. Growth and mortality of chicks from eggs laid by hens being fed 5.0 ppm Se were not affected, whereas those from hens on a diet containing 10 ppm Se had a greatly increased mortality rate. Diets containing 5 and 8 ppm Se from grain had no effect on growth of chicks while diets containing 10 ppm caused a decrease in growth rate (Poley et al. 1941). In the same study, it was demonstrated that feeding 4.0 ppm Se but not 2.0 ppm in the dam's diet decreased growth in the chicks when they were grown on a diet with no seleniferous grain. Poley et al. (1937) previously had shown that laying hens fed 15 ppm selenium from grain ate less and lost weight but egg production and fertility were not affected. However, egg hatchability was reduced to zero because of deformed embryos.

Laying hens were fed graded levels of sodium selenite by Ort and Latshaw (1978) and they found that egg production, weight and

fertility were not affected by up to 5 ppm Se. Hatchability was reduced by 5 ppm, egg weight was reduced by 7.0 ppm and egg production was reduced by 9.0 ppm. Thapar et al. (1969) found that 2.0 ppm dietary selenium as sodium selenite had no effect on laying hens or their eggs, but 8.0 ppm decreased body weight, egg weight, production and hatchability, and progeny growth.

When eggs from hens fed 8.0 ppm selenium from seleniferous wheat were incubated, Gruenwald (1958) found necrosis in certain areas of the brain, spinal cord, eyes and limb buds after 2.5 to 3 days. By 5 days, there were defects in the face, nasal pits, upper beak and caudal portions of the embryo as well as growth inhibition.

Carlson et al. (1951) have shown that 20 ppm Se in the diet of growing turkeys was required to produce toxicity signs. However, 9 ppm selenium in the diet of laying turkeys produced some malformations in embryos and at 15 ppm in the diet, almost all embryos were malformed. Recently, Cantor et al. (1984) have reported that 2 ppm in the drinking water of Single Comb White Leghorn chicks had no effect on growth.

In studies with swine, Miller and Schoening (1938) fed 17 kg animals a diet containing 0, 24.5, 49, 196 or 392 ppm selenium as sodium selenite. Most pigs showed typical signs of "alkali disease" including loss of appetite, loss of hair and coronary band lesions. All pigs died in from 10 to 99 days. Moxon (1941a) found that feeding a pig a diet containing 9.0 ppm Se from corn caused a reduced weight gain, hoof lesions, roughing of skin and loss of hair in about 20 weeks.

It is difficult to assign the lowest level of dietary selenium that causes a measurable response in the performance of swine because of the varied experimental conditions used. Schoening (1936) found that on a diet of corn alone, containing 5 or 10 ppm of naturally occurring selenium, only those pigs fed the 10 ppm level developed signs of toxicosis. Wahlstrom et al. (1955) showed that 7.0 ppm Se as selenite, fed in a corn-soy type of diet, caused signs of chronic selenosis. In the same study, 10 ppm caused emaciation and reduced weight gains. These workers noted that red pigs (Duroc) were more susceptible to selenosis than were the black and white pigs (Poland China). Much more recent work by Wahlstrom et al. (1984) has shown similar findings. Young weanling pigs, 10 each of red, white and black pigs, were fed a corn-soy diet with 8 ppm selenium added as sodium selenite. The black and white pigs showed no typical signs of toxicosis other than a small reduction in rate of gain, whereas the red pigs had a greatly reduced rate of gain. In addition, two of the red pigs developed paralysis of the hind quarters and 4 had hoof lesions. On the other hand, after 35 days on experiment, the red pigs had the least selenium in their

hair (3.7 ppm versus 12.2 ppm for the black and 10.5 ppm for the white).

In another study, Wahlstrom et al. (1956) reported the presence of the typical signs of toxicosis in pigs on a diet containing 13 ppm Se as selenite but that the rate of gain of the pigs responded quickly to removal of the selenium from the diet. Wahlstrom and Olson (1959) fed a diet containing 10 ppm Se as selenite to sows and observed the following symptoms: loss of hair, hoof lesions, reduced gain in weight, lowered conception rate, increased number of services per conception, a higher percentage of dead pigs, smaller and weaker pigs at farrowing and fewer and smaller pigs at weaning. Weanling pigs from these sows gained less than pigs from sows on a diet without added selenium.

There seems to be little question about the toxic effects of dietary levels of 10 ppm Se or greater. Herigstad et al. (1973) fed 4 week old pigs diets of the Torula yeast or whole milk powder type containing graded levels of sodium selenite or selenomethionine up to 600 ppm Se. All pigs on diets with 20 ppm Se or more showed some signs of toxicosis. Signs were similar for both forms of selenium although CNS lesions were more severe for the selenite. Wilson et al. (1982) reported an accidental case of selenium poisoning in swine on a diet found to contain about 54 ppm Se. Paralysis was observed in 18 of 100 pigs in 8 to 10 weeks of age. Histologically, they found symmetrical focal poliomyelomalacia. The workers were able to reproduce the syndrome in growing pigs by feeding a diet with 50 ppm Se a selenite. More recently, Wilson et al. (1988) have produced the syndrome in one out of four pigs with a daily encapsulated dose of 1.4 mg sodium selenite (0.64 mg Se)/kg body weight. Assuming an average body weight of 10 kg and a food consumption rate of 2.5% of body weight, the encapsulated dose would be equivalent to consuming a diet containing 25.6 ppm Se. Harrison et al. (1983) have reported three instances of accidental selenium poisoning in swine. Feed collected from two of the cases contained 10 to 24 ppm Se. This feed was fed to 10 pigs weighing about 16 kg and produced many signs of selenium toxicity including paralysis in two pigs. Thirteen cases of paralysis were noted in the original field cases.

Mahan and Moxon (1984) fed weaned pigs for 37 days on a corn-soy diet with added sodium selenite at levels of 0, 2.5, 5.0, 7.5, 10, 15, 20 or 40 ppm Se. No toxic effects were noted in the pigs on the diet containing 2.5 ppm Se, but those on the 5.0 ppm Se diet showed some reduction of gain in weight. Alopecia was also noted in pigs fed diets with more than 5.0 ppm Se, and reduced gain in weight was observed at all levels of 7.5 ppm and above. Abnormal hooves, anorexia, and lethargy were also observe at the 7.5 ppm level and above.

Goehring et al. (1984) fed a grain-soy type diet containing about 0.5, 2.6, 5.6 or 8.4 ppm Se as sodium selenite or seleniferous wheat and oats to weanling pigs and weanling rats. No obvious signs of toxicity were noted based on rate of gain and certain blood values for the pigs. The same diets appeared to be slightly more toxic to rats. In a subsequent study, Goehring et al. (1984a) fed weanling pigs a corn-soy type diet with 0, 4, 8, 12, 16, and 20 ppm added Se as sodium selenite. By 5 weeks, growth data suggested that the toxicity of selenite for this type of diet lay between 4 and 8 ppm Se. Two pigs on the diet containing 12 ppm Se developed hoof lesions and one pig on the 20 ppm Se diet developed paralysis of the rear quarters.

STUDIES WHICH APPARENTLY SHOW A UNIQUELY GREAT TOXICITY OF SELENIUM

Although most of the studies in the literature on animals do not report toxic effects of selenium if present in the diet at less than 1.0 ppm, there are a few notable exceptions that will be discussed here. As stated in a previous section, Ermakov and Kovalskij (1968) have reported signs of chronic toxicity in sheep which consumed feeds containing 2 mg Se/kg (fresh weight). The signs observed were hoof deformation, loss of hair, hypochromic anaemia and increases in alkaline and acid phoshatases in various tissues. The original article is in Russian and the authors of this review have not seen the data (obtained from WHO, 1987). This is much greater toxicity than reported by any others workers and needs to be validated.

Tinsley et al. (1967) and Harr et al. (1967) carried out an extensive study on the chronic toxicity of selenium in rats. They used 1437 Wistar rats from a closed random bred colony. Experimental groups varied from 10 - 110 animals in a rather complicated design. Three diet types were fed including a semi-purified diet containing 12% or 22% casein and a commercial laboratory chow. Selenium as sodium selenite or sodium selenate was added at 0.0, 0.5, 2.0, 4.0, 8.0 or 16 mg/kg diet. Very few animals survived for 12 months with supplementation of 4.0 mg Se/kg or greater. The rats on commercial diets were 2 to 3 times more resistant to effects of selenium toxicity than those on purified diets. A calculated maximum body weight was reportedly depressed by as little as 0.5 mg Se/kg diet, but no statistical evaluation of the data was given. Harr et al. (1967) also reported an increased proliferation of the hepatic parenchyma when rats were fed the semipurified diets supplemented with even the lowest level of selenium (0.5 mg Se/kg diet). However in a more detailed report of the same experiments, Weswig et al. (1966) showed that this lesion of "Chronic liver and bile duct hyperplasia" was observed to a greater extent in rats fed the commercial diet which was not supplemented with selenium. Therefore, this lesion is probably not specifically related to selenium.

A recent review article (WHO, 1987) cites a paper by Harr and Muth (1972 p.101) as stating that 0.25 mg Se/kg diet was the minimum toxic level for liver lesions when selenium was added to semi-purified diets and that 0.75 mg Se/kg diet was the minimum toxic level when longevity, or lesions of the heart, kidney or spleen were the criteria of toxicity. It should be noted that the paper by Harr and Muth (1972) is a review and the estimations are made from the same data as reported by Tinsley et al. (1967) and Harr et al. (1967) and it is unclear how the estimates for minimum toxic levels were derived since selenium was not supplemented at levels lower than 0.5 mg/kg. As stated before, no statistical evaluation of the data was performed. These

estimates were not made in the original article. It is also of interest that the review article (Harr and Muth, 1972) states that growth was not inhibited by supplementation with 0.5 mg Se/kg diet and this seems to contradict the original work of the authors (Harr et al. 1967).

Csallany et al. (1984) have reported that giving sodium selenite to female mice at a level of 0.1 mg Se/L increased the amount of hepatic lipid soluble lipofuscin pigment at 9 months of age. The significance of this report is not understood.

Pletnikova (1970) investigated the effects of long-term, low-level administration of sodium selenite in drinking water to 32 rabbits and 16 rats which were divided into groups of 4 animals each. Animals were given daily oral doses at rates of 5, 0.5, or 0.05 μg Se/kg of body weight for 7.5 and 6 months, respectively. Many diagnostic tests were conducted. Especially at the 5 μg Se/kg of body weight rate of administration, changes were noted in the rabbits in many of the parameters measured. Selenite was used in the study, since it was found to be more toxic than selenate. By olfactory and organoleptic tests, an odor had been noted in waters containing 10 or 25 μg Se/L, and an astringent taste was found in waters containing 100 μg Se/L.

A daily dose of 5 μg Se/kg of body weight has been calculated to be the equivalent of about 63 μg Se/L, and thus is well within the physiological range needed to prevent selenium deficiency in animals, and the toxicological significance of Pletnikova's findings is not clear (WHO 1987, P 102). None-the-less, they were used as a basis for her recommending 1 μg Se/L as the maximum permissible concentration for drinking water for man.

Palmer and Olson (1974) found that rats could tolerate up to about 2000 μg Se/L as selenite or selenate in their drinking water for periods of at least 8 weeks with only a slight decrease in average daily gain and feed and water intake. Selenite seemed slightly more toxic that selenate. Rosenfeld and Beath (1954) reported that selenium concentrations of 1.5 or 2.5 mg/L in the drinking water of rats had no effect on reproduction, although 2.5 mg Se/L did reduce the number of young reared by the second generation of mothers.

In unpublished research at the South Dakota Agricultural Experiment Station, the effects of various concentrations in the drinking water of male, albino rats (Sprague-Dawley) weighing about 70 grams at the outset was studied. The rats were individually housed in stainless steel wire cages (18 x 18 x 25 cm, hwd) and fed a torula yeast-glucose diet (Halverson 1974). Water was offered continuously in two bottles at the front of the cages. One contained deionized water (control), the other

(treated) deionized water with 0.0, 12.5, 50, 200, 800, or 3200 μg Se/L (as potassium selenate). The water bottles were checked daily, and refilled from a refrigerated stock prepared weekly and stored at 0-4 C. Water consumption for each rat was measured and recorded. After the third week, the bottles containing the control and treated waters were reversed. This relocation was made because it had been noted in preliminary work that placement of the bottles did affect usage.

At six weeks, the rats were weighed and the experiment was concluded. The findings are summarized in Table 2-4. They indicate that only at the 800 and 3200 μg Se/L concentration did the rats avoid the treated waters. At the 800 μg Se/L

Table 2-4 The ability of rats to discriminate between waters of different selenium concentrations.

Se concentration in the water (μg/L)	Average initial weight of rats (g)	Average daily gain of rats (g)	% of total water intake from control bottle (at 6 wks.)
0	68	6.4	53.6
12.5	71	6.8	47.7
50	74	6.3	58.2
200	71	6.9	58.2
800	71	6.7	83.7 (c)
3200	68	6.8	94.4 (c)
	(a)	(b)	

(a) "F" value 0.76 by ANOVA ns
(b) "F" value 0.84 by ANOVA ns
(c) "F" value 17.20 by ANOVA ($P<0.01$). Significantly different ($P<0.01$) from control (0.0 μg Se/L by LSD protected by a significant "F" value).

concentration, avoidance was significant during the second and all succeeding weeks ($P<0.01$ by LSD), and it was significant ($P<0.01$) during all six weeks of the experiment at the 3200 μg Se/L concentration. As might be expected, there was no significant difference in average daily gain of the rats. Being allowed a choice of waters the rats were able to discriminate between those of low or of high selenium concentrations, thus restricting their intake of the element in the waters of the higher selenium contents. A similar ability to discriminate between diets of varying selenium contents has been reported by Franke and Potter (1936) for rats. There is nothing in the study reported in Table 2-4 to suggest that either odor or taste would

reduce the intake of water with the very low selenium contents used by Pletnikova. Nor do the data indicate the need for a more restrictive maximum permissible level of selenium in drinking water for man than 10 μg Se/L.

Although not shown in the table, the data for consumption from the bottle on the right versus the bottle on the left gave no significant difference ($P>0.05$) for any treatment or week. The overall average for intake from the bottle on the right being 48.2 ± 5.3.

Although it is proper to keep the above reports of excessive toxicity in mind, there is question as to the validity of some of them and question about the physiological significance of others. It appears that the bulk of the literature does not support the reported effects of such low dosages of selenium as those reported by Tinsley et al. (1967), Csallany et al. (1984), and Pletnikova (1970). The acceptance of such data awaits verification by other well conceived and well executed experiments.

SUMMARY OF THE LITERATURE RELATIVE TO THE TOXICITY THRESHOLD DOSE AND THE NO EFFECT DOSE

Because of the number of variables involved, it is very difficult to establish a single value for the minimum amount of selenium necessary to produce toxic signs and the maximum level of selenium that will produce no signs of toxicity. However, in reevaluating the literature reviewed in recent publications (NAS 1972; Olson 1986; WHO 1987) and the literature which has appeared since the publication of the reviews (essentially articles in 1986 to 1988), it is the opinion of the authors of this review, that the level of 4 - 5 mg Se/kg diet (0.1-0.125 mg Se/kg body weight) is still an adequate estimate of the level at which toxic signs most likely will appear.

Relative to the maximum selenium dietary level which will not produce signs of toxicity, it appears that a dietary level of 1 ppm (0.025 mg Se/kg body weight) is a reasonable estimate. This assumes the animals are in good health and are being fed a nutritionally balanced diet. This estimate is based on the fact that several studies report no signs of toxicity at 2.5 ppm Se. The level of 1 ppm Se in the diet has been suggested as safe for humans by Olson (1986), and others have suggested levels of the same magnitude (McCarty, 1984; Sakurai and Tsuchiya, 1974).

EVALUATION OF NEED FOR SEPARATE SELENIUM GUIDELINES FOR EACH CLASS OF LIVESTOCK

It is very likely that the toxicity of selenium differs for the various classes of livestock. Data to accurately quantitate the differences are, however, not available. One reason for this lack of data is that so many factors affect the element's toxicity. Experimental work to provide an estimate of the toxicity that takes the many variables into account becomes, therefore, excessively expensive. Further, additional work might not sufficiently improve our capacity to arrive at a valid criterion to warrant the expense.

Examples of the factors needing consideration are: climate, sex of the animals, breed, diet, activity of the animal, meat or milk production, or egg production. Of the variables that affect selenium's toxicity or its accumulation in animal products used for human food, few have more than a minor effect. Thus it seems unlikely that extensive studies to develop maximum allowable concentrations for each class of livestock would prove of much value over what good judgment in the use of data now available would provide.

THE BIOACCUMULATION OF SELENIUM IN LIVESTOCK TISSUES OR FOOD PRODUCTS

The Committee on Medical and Biologic Effects of Environmental Pollutants reported that there is little evidence to indicate any biomagnification of selenium in the food chain (NAS-NRC 1976, p. 147). While this may be true with respect to some food chains, it is now well accepted that for fish and some aquatic birds and some wildlife, very significant bioaccumulations of the element can occur. Therefore, the possibility that its accumulation in livestock tissues used as human food cannot be disregarded. Unfortunately, there are few data dealing with the latter issue. Some examples of the kind of information that is available are discussed below.

Goehring et al. (1984) fed pigs diets containing up to just over eight ppm of selenium from seleniferous grain or from sodium selenite and analyzed some of the tissues at six weeks. Using their data corrected for moisture content of the feed and of the tissues, accumulation factors were calculated as indicated in Table 2-5 (ratios of Se content of dry tissue to Se content of dry feed of greater than 1.0 indicate bioaccumulation). The data clearly show that the selenium from grains accumulates in animal tissues to a greater extent than does that from sodium selenite, that the kidney and liver accumulate considerably more than does muscle, and that the accumulation factor increases with increasing selenium content of the diet. The muscle tissue appears quite resistant to elevation of Se level by oral ingestion of inorganic Se. Since the form of selenium in waters of high selenium content is normally almost entirely in the form of selenite and/or selenate, bioaccumulation from waters, especially those containing less than 0.050 mg/L, is unlikely to be a problem.

Data (Olson and Embry 1973) from a heifer fed a diet containing 20% alfalfa hay, 74% rolled corn, 5% soybean meal, 0.5% dicalcium phosphate, 0.5% trace mineral salt, and 15 mg Se/kg as selenious acid for 231 days were used for similar calculations as above, and the selenium contents and accumulation factors, respectively were: for liver, 28.5 mg/kg and 1.9; for muscle, 1.8 mg/kg and <0.1.

Conrad and Moxon (1979) fed dairy cattle a feed with various concentrations of added selenite up to 0.5 mg Se/kg and concluded that the small amounts transferred to the milk were not a potential hazard to human health. Maus et al. (1978) concluded from their studies with dairy cattle that dietary selenium up to 0.7 mg/kg as selenite should not result in toxic amounts of the element in the milk.

Table 2-5 Bioaccumulation of selenium in swine tissues[a].

Source and amount of Se in diet		Selenium content of tissues of the animals mg Se/kg dry basis[b]			Accumulation factor for tissues[c]		
		Liver	Kidney	Muscle	Liver	Kidney	Muscle
Grain	2.87 mg/kg[d]	10.6	17.2	4.0	3.7	6.0	1.4
	6.22 mg/kg	17.1	25.6	7.0	2.8	4.1	1.1
	9.33 mg/kg	22.6	33.1	9.3	2.4	3.5	1.0
Na_2SeO_3	2.92 mg/kg	8.5	10.8	0.9	2.9	3.7	0.3
	6.32 mg/kg	10.7	12.2	1.0	1.7	1.9	0.2
	9.25 mg/kg	12.1	14.9	1.2	1.3	1.6	0.1

[a] Values calculated from data of Goehring et al. (1984).

[b] Calculated from data for undried tissues assuming a moisture content for liver as 70%, kidney as 80%, and muscle as 60%. These percentages approach the values given in USDA (1983).

[c] Selenium content of the dry tissues divided by selenium content of dried feed. Only those values of greater than 1.0 indicate bioaccumulation.

[d] Selenium content of the feed on a dry basis. In calculating this, it was assumed that the feeds contained 10% moisture.

Data from Carlson et al. (1962 for growing chicks on diets containing 10 mg Se/kg as selenite or 8 mg Se/kg from grains calculated in a manner similar to that described above, gave accumulation factors as follows: (selenite treatment) liver, 0.5 and muscle, 0.15; (Se grain treatment) liver, 2.5 and muscle, 1.8.

Arnold et al. (1973 reported data on eggs from hens fed a corn-soy diet. At 62 weeks on experiment, hens on the diet containing 2 mg Se/kg as added selenite contained 0.16 mg/kg more than the controls (no added selenite) giving an accumulation factor of less than 0.1, while those on the diet with 8 mg added Se/kg contained 1.38 mg Se/kg more than the controls, giving an accumulation factor of 0.2. It appears that eggs from hens drinking waters containing 0.05 mg Se/L should not contain excessive concentrations of the element.

No further effort to make similar calculations from data in the literature will be attempted, although many more ar possible. For instance, examination of available data for laboratory animals such as the rat (Palmer et al. 1983; Goehring et al. 1984) would lead to similar conclusions relative to muscle tissue accumulation of selenium. It appears obvious that the prevailing forms of the element in water have little tendency to accumulate in livestock tissues at dangerous concentrations when the level of 0.050 mg Se/L is not exceeded, that the food chain from water to livestock tissues is a short one, and that the statement by the Committee on Medical and Biologic Effects of Environmental Pollutants (NAS-NRC 1976, p.147), relative to the lack of biomagnification of selenium in the food chain, is correct.

JUSTIFICATION FOR THE CURRENT GUIDELINES FOR SELENIUM CONTENT OF DRINKING WATER FOR ANIMALS

For man, the recommended maximum allowable concentration for a safe level of selenium in the drinking water in the United States has changed somewhat over the past half century. For instance, the U. S. Public Health Service recommendations have changed as follows (McKee and Wolf 1963, p.91):

Year	Recommended Standard
1925	None established
1942	50 μg Se/L
1946	50 μg Se/L
1962	10 μg Se/L

At present, the recommendation is still 10 μg Se/L, as recommended by the EPA Committee on Water Quality Criteria (NAS-EPA 1972, pp.304-22). The WHO International standard for selenium in drinking water for man was 50 μg Se/L for 1958 and for 1961. There seems to be no compelling data that would direct the adoption of more restrictive guidelines for man.

The 1972 NAS-EPA guidelines for levels of selenium in animal drinking water state that 0.05 mg/L is the maximum acceptable concentration. In reviewing the literature relative to selenium toxicity, it would appear that much of the data support the assumption that no observable signs of toxicity will be produced by up to 1 ppm in the feed. Translating selenium intake from this dietary level into a drinking water concentration that will give an equivalent intake is complicated by several factors. These include the estimation of an average feed consumption and water intake. The water intake, in turn, varies greatly depending on the animal species, ambient temperature, quality of water and type and amount of feed intake. The calculations and estimations which follow, are based on a "worst case" scenario.

Of major concern in establishing drinking water guidelines is the water intake of the animals. The intake may be greater than the requirement and it may be greatly influenced by temperature, lactation, salt content of the water, etc. Church and Pond (1988) make the generalization that animals will consume 3-4 grams water for every gram of dry feed when they are not heat stressed. Species with the capability to conserve water, such as sheep, will require less while cattle will probably require the most. Birds generally require less water than mammals and young animals will usually require more water per unit of body weight than adults. It appears that animal nutritionists commonly assume a ratio of water to feed intake of about 3:1.

The data presented in Tables 1-1 and 1-2 show that considerably higher water consumption can occur, particularly with cattle. This is an important factor in considering what the maximum allowable selenium content in water should be.

The data of Winchester and Morris (1956) (also shown in Tables 1-1 & 1-2) were used to calculate water to feed intake ratios for certain dairy and beef cattle at two different temperatures. These calculations are shown in Table 2-6. The data show that at 4°C, the water consumption was such as to give a water to feed ratio of about 3:1. However, at 32°C the water

Table 2-6 Ratios of water to feed intakes for cattle calculated from data of Winchester and Morris (1956)

Class of Cattle	Kg water:Kg feed	
	4°C	32°C
Dairy cattle		
Heifers	3.00	7.35
Bulls	3.08	7.34
Non-lactating cows	3.09	7.34
Beef cattle		
On maintenance diet	3.09	7.34
Bulls	3.09	7.33
Cows on hay and/or grain		6.42
Cows on high-salt diet		9.22

to feed ratio was almost 7.5:1. No doubt this temperature represents a high heat stress, since the animals were held at a constant temperature which would be much higher than the average daily temperature they would be exposed to in areas such as the San Joaquin Valley. It does represent the extreme in water intake by animals. A ratio of 5.1:1 obtained by Ray (1989) in a study conducted in Arizona might represent a more likely estimate of ambient conditions in the San Joaquin Valley.

It is reasonable to assume that animals would be able to safely consume the same amount of selenium in drinking water that is allowed to be added to feed. The FDA currently has approved the addition of 0.3 ppm selenium to feeds as inorganic selenium (FDA, 1987). Assuming the extreme water to feed intake ratio of 7.5:1, water containing a concentration of 0.040 μg/mL (0.3

divided by 7.5) would provide the same selenium intake as 0.3 ppm added to feed.

Another way of looking at the same problem is to calculate the selenium intake from the maximum expected water consumption for various adult livestock of medium weight at a temperate climate. The results of these calculations are shown in Table 2-7. The calculations are made on the basis of assumed body weights of various animals and on the assumption that animals consume feed at an average of 2.5% of body weight. Since the maximum value for expected water consumption is used, these calculations also approximate a "worst case" situation. The fifth column of Table 2-7 gives the selenium intake from the water, assuming it contains the currently accepted maximum selenium concentration of 0.05 μg/mL. Dividing this value by the estimated feed intake for each animal gives the concentration of selenium in the diet that would provide an equivalent daily intake (shown in the last column). These values can vary from 0.18 μg Se/g diet to 0.49 μg Se/g diet, with an overall average of 0.35 ± 0.13. This agrees quite well with the calculated dietary level from the other worst case study involving heat stressed animals.

Table 2-8 presents a summary of the comparative calculations of selenium intakes from situations of various selenium concentrations in feeds and water. At the extreme water to feed ratio of 7.5:1, the amount of selenium intake by animals drinking water containing the maximum accepted selenium concentration would be equivalent to consuming a level of 0.375 ppm selenium in the diet. This is slightly above the level that has been approved for supplementation (0.3 ppm). However, it is still one half of the level considered to give no observable toxicity signs and it is one tenth of the level considered to produce toxicity. Choosing a water to feed ratio of 7.5:1 is certainly a worst case situation, since animals would rarely be at a temperature of 32°C for long periods of time. The level of intake from water under these extreme conditions is very close the that provided by the approved supplementation of the diet with 0.3 ppm. Therefore, the current guideline of 0.050 μg/mL in animal drinking water seems to be justified and probably represent a conservative and rational limit.

In support of this conclusion is the observation that animals consuming water at this level would consume the amount of selenium equivalent to that supplied by 0.375 ppm in the diet. If it is assumed the diet contains 0.4 ppm natural selenium, in addition to the permitted 0.3 ppm supplemental selenium, the total selenium intake would be equivalent to that from a dietary concentration of 1.08 ppm. This is close to the level of no effect. The average selenium content of the feeds in the San Joaquin Valley are usually less than 0.4 ppm used in the above calculation (Kubota et al. 1967; Burau et al. 1987).

Table 2-7 Daily Se intake calculated from maximum expected intake of water containing the accepted limit of 0.05 ug Se/mL

Animal	Typical body wt.	Feed consumed (2.5% body wt)	Maximum expected water intake[a]	Se intake from water containg 0.05 mg/mL	Dietary Se content to provide equivalent Se intake as in water
	Kg	Kg	L/day	mg	mg/Kg
Beef cattle	363	9.1	72	3.6	0.40
Dairy cattle	450	11.3	110	5.5	0.49
Swine	113	2.82	19	0.95	0.39
Horses	454	11.4	45	2.25	0.20
Chickens	2.3	0.058	0.4	0.020	0.34
Turkeys	6.8	0.17	0.6	0.30	0.18
				Average ± SD	0.35 ± .12

[a]Taken from NAS-NRC 1974

It should be emphasized that the worst case situation has been used to make the above calculations. If one assumes the more accepted average water to feed ratio of 3:1, then the amount of selenium contributed by water containing 0.05 μg/mL would be equivalent to 0.15 ppm in the diet. This intake from water would supply only one half of what is currently permitted for feed supplementation. **In any event, the current guideline is appropriate relative to safety for animals.**

Table 2-8 Some calculations of selenium intakes and toxicities in feeds and waters

	Feed content mg Se/Kg diet	Equivalent drinking water content[b] mg/L	Animal dosage[c] μg Se/Kg bw/day
	(A)	(B)	(C)
Threshold Toxicity	4-5	0.53 - 0.66	0.10 - 0.125
"No effect" dose	1	0.13	0.025
FDA recommended level of diet supplementation	0.3[d]	0.04	0.0075
NAS-EPA guideline for maximum Se content of livestock drinking water	0.375	0.05[e]	0.0094

[a] In the first three rows, Col. A/7.5 = B and A x 0.025 = C. In the fourth row, Col. B x 7.5 = A and B/7.5 = C.
[b] Assuming a 7.5:1 water to feed intake ratio
[c] Calculated assuming a feed consumption of 2.5% body weight
[d] FDA 1987
[e] NAS-EPA 1972

RECOMMENDED GUIDELINE FOR THE SAFE LEVEL OF SELENIUM IN ANIMAL DRINKING WATER

The currently approved guideline for the safe level of selenium in animal drinking water is 0.05 mg Se/L (NAS-EPA 1972). The previous discussion has provided validation that this level provides no more selenium per day to the animal under maximum water intake conditions than does the currently approved level of dietary supplementation. The approved dietary supplementation guidelines have recently been raised from 0.2 ppm to 0.3 ppm to obtain maximum positive physiological response. This was done after many years of experience in the field in which no deleterious effects were noted. Therefore, the appropriate safety factor seems to be built into this level of supplementation. Although studies on the comparative toxicity of selenium in the drinking water and feed of farm animals is lacking, the data from laboratory animals, which include selenium intake measurements, do not indicate a difference of meaningful magnitude. **Therefore, it is the opinion of the reviewers that the current guideline for selenium in livestock drinking water of 0.05 mg Se/L, is adequate and safe. There do not seem to be compelling reasons to change it at present.**

The reports on the toxicity of selenite or selenate selenium do not clearly establish which is the most toxic, although the preponderance of data suggest it is the selenite. These two forms, and especially selenate, comprise the major portion of the total soluble selenium in waters potentially toxic. It seems, therefore, that an analysis for total soluble selenium should be accepted as determining whether a water meets the 50 mg Se/L standard.

In the event that regional guidelines are ever adopted, there would be justification for changing the current guidelines for certain regions of the nation. For instance, if the selenium level in feed is taken into consideration, the fact that the normal level of selenium in feed in the San Joaquin Valley is probably about 0.1 ppm would allow an intake from water to be twice what is now permitted (equivalent to 0.100 mg Se/L). In addition, if a regional potentially usable source of water with known elevated levels of selenium is available such as is the case with irrigation drainage, feed supplementation could be curtailed. If this were done, a drinking water level of 0.100 mg Se/L would provide the same level of selenium as obtained with the feed supplement plus drinking water with the currently allowed maximum selenium level. If one combines both the curtailment of feed supplementation and the consumption of feedstuffs of low selenium content (<0.1 ppm), then a level of 0.150 mg Se/L in the drinking water would be acceptable. Without such graded or regional guidelines, it appears the currently recommended maximum level of selenium in drinking water of 0.05 mg Se/L is a safe and rational overall guideline.

RECOMMENDATIONS FOR FUTURE STUDY

In attempting to make recommendations concerning a guideline for safe levels of selenium in livestock drinking waters of the San Joaquin Valley, it became obvious at the outset that data relative to long-term effects of the lower concentrations of the element on farm animals are not plentiful. Yet, these are the data most valuable in establishing the guidelines. It is, therefore, important that research using livestock instead of experimental animals such as rats be undertaken to fill this gap. Since it would hardly be either essential or highly fruitful to work with all classes and sizes of animals, or to study all effects of the various factors that might affect the toxicity of selenium, it is suggested that studies be limited to cattle, swine, and poultry, that they be conducted over at least a three-year period, that they be designed to allow for some temperature effect calculations from the data obtained, and that they include reproductive performance. It should be recognized, in planning and performing the work and in interpreting the results that unless a harmful effect is obtained at the highest concentration(s) used, the recommended safe level may be set at a concentration lower than necessary. Thus a number of waters might be precluded from use even though they did not have excessively high selenium contents. In the use of the value selected as the uppermost safe concentration, it must be recognized that this value may be exceeded without meaning that the animals drinking the water are necessarily being poisoned.

As part of any study on this matter, not only gross signs of toxicity should be observed, but histopathologic changes as well. With reference to the latter, if changes are noted, then the significance of these changes to the health of the animal should be explained.

An experimental design similar to that used by Winchester and Morris (1956) in studying the temperature effect on water consumption, substituting water selenium content for temperature might be considered. Selenium concentrations between about 130 μg/L and 530 μg/L should be used, since these probably lie very close to the "no effect" selenium content of the water and to the "threshold toxicity".

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CHAPTER 3: GUIDELINES FOR ALLOWABLE LIMITS OF MOLYBDENUM IN DRINKING WATER FOR ANIMALS

Gerald M. Ward

Department of Animal Science
Colorado State University
Fort Collins, CO 80523

INTRODUCTION

Molybdenum (Mo) is widely distributed in nature. Its average abundance in the earth's crust is about 1 mg/kg or 1 part per million (ppm). Mo is one of the most highly concentrated trace elements in sea water at 10 parts per billion (ppb) (Hem, 1985).

A recent one-time survey of 212 streams in California by the Central Valley Regional Water Quality Control Board showed background molybdenum concentrations exceeded 5 μg/L in almost 25% of the streams tested and 14% of the streams tested showed molybdenum concentrations in excess of the 10 μg/L standard considered safe for water being used to irrigate forage and pasture (Westcot, 1989). Mo is frequently found in shallow groundwater through the San Joaquin Valley. Concentrations have been found to vary from 5 μg/L to >7,500 μg/L with the most highly concentrated samples found in the lower San Joaquin Valley. The occurrence of molybdenum in groundwater in the San Joaquin Valley is highly associated with the geological setting. The highest concentrations are found in the lake bed and basin rim deposits of the Valley (Westcot et al., 1988a; Chilcott et al., 1988). In the lower San Joaquin Valley, high concentrations of molybdenum in shallow groundwater are associated with strongly elevated concentrations of uranium (Westcot et al., 1988b).

Molybdenum has been shown to be a required element for both plants and animals. Mo is a required element in three enzyme systems in the animal body; xanthine oxidase, aldehyde oxidase and sulfite oxidase. A Mo deficiency has been described in patients parentally fed that was corrected by Mo supplementation (Mills and Davis, 1987). No deficiencies have been associated with normal diets although in an area of China a high incidence of esophageal cancer is associated with food of low Mo content (Luo et al., 1982).

No naturally occurring Mo deficiency has been reported for animals. Rats and chickens have shown no adverse effects from diets containing 0.2 ppm of Mo. A significant growth response to Mo was shown in lambs fed a semi-purified diet containing 0.36 ppm (Mills and Davis, 1987). However, many pastures are known to

contain less than this amount of Mo. Anke et al. (1985) states that the Mo requirement for goats is 100 micrograms per kg of feed or 0.1 ppm which may be a reasonable estimate for ruminants. In Norway, forage very low in Mo (<0.3 ppm) and containing moderate levels of Cu is suggested to be responsible for Cu toxicity in sheep (Norheim and Froslie, 1985) and growth improved with Mo supplementation.

The practical significance of Mo in the environment is its potential to produce toxicity particularly in cattle and sheep. The National Research Council Committee in 1974 concluded that data available did not warrant establishing a safe upper limit for Mo intake by animals. A report for EPA (Feiberg et al., 1975) came to no conclusion about a safe limit of Mo intake for humans. The Environmental Protection Agency proposed (October 1983) discussion of guidelines for Mo in drinking water for humans and suggested 0.05 ppm as the limit based upon a suggestion by Chappell (1979) who had concluded that 0.05 ppm "was prudent" because "no biochemical changes were found in subjects consuming water containing up to that level." Gilliland (unpublished report) contends that the research data on humans suggests that 2 to 5 ppm in human drinking water is safe and a minimal concentration of Mo is necessary to prevent certain cancers. EPA has deferred any decisions on guidelines.

The objective of this paper is to review the available information and develop guidelines for Mo content in drinking water that will assure the safety of animals consuming it. Although the subject to be addressed is the safe level in water, essentially all of the information available concerning the effects of Mo on animals relates to Mo in forages or as supplemental molybdate salts added to feeds.

The question arises as to possible differences in availability of Mo in plants or feeds as compared to the soluble form found in water. There is only one comparison between Mo in water and Mo added to feed. Absorption appeared to be lower in rats when Mo was incorporated into feed than when given in water (Winston et al., 1985). These results for rats probably are not relevant to ruminants because the reducing conditions in the rumen and the production of the molybdates is believed to be responsible for the greater toxicity of Mo to ruminants. A study of Mo added to the water supply of calves at 10 ppm showed no effect and 50 ppm only a limited effect. The calves diets contained 13 ppm of Cu and 0.29% S. The calves, however, were recently weaned and it is not clear to what extent they had functional rumens. The authors (Kincaid, 1980) suggested that Mo in water might be less toxic than in feed because they expected toxic effects at these levels. The Mo consumed by livestock in water or feed is in the form of soluble molybdate anions (James Gilland, Personal Communication).

In order to establish a safe level of Mo in drinking water it is necessary to review the literature on animal responses to Mo in feeds. In addition to water intake of Mo it will be necessary to know what Mo is contributed by feed, the interaction of other minerals with Mo and the influence of feeding practices upon Mo metabolism.

MOLYBDENUM TOXICITY

Molybdenum toxicity was first identified in 1938 as the cause of a long recognized problem of severe diarrhea and emaciation in cattle grazing certain areas called "teart" pastures in Somerset County in England. In the same year it was reported that the problem could be corrected by feeding copper sulfate (Ferguson et al., 1938). In the San Joaquin Valley of California similar symptoms had been reported in cattle since 1860 and it was attributed to excess Mo in the forage when the similarity to the cases in England was recognized. Molybdenosis problems in California are found in alkali soil areas especially on the west side of the San Joaquin Valley (Clawson, 1973). Similar areas with Mo problems were later identified in areas of Nevada (Dye and O'Hara, 1959). Chronic Cu deficiency symptoms also have been reported in grazing cattle from southern Oregon and western Manitoba associated with low Mo levels in forage. Kubota (1977) has surveyed the U.S. for Mo levels in soils or plants and above normal levels generally correlate with observed Mo toxicity areas. Alloway (1977) points out that although plant uptake of Mo is greater from alkaline soil the majority of Mo toxicity areas of the world are associated with low-lying wet soils. He feels that wet soils are more indicative of Mo problems in livestock than alkaline soils. Ward (1978) reviewed reported molybdenosis cases related to mine (especially uranium mines) and industrial contamination of grazing lands and found that, although Mo was probably the causative agent in most cases, the data was inadequate to relate Mo intake to the clinical signs reported.

The clinical signs of Mo toxicity or molybdenosis are primarily due to a conditioned or secondary Cu deficiency and the problem can be further exacerbated by increased S in the diet. The first symptoms of severe Mo toxicity in cattle is a debilitating diarrhea leading to emaciation, loss of weight and sometimes death. Anemia due to deficiency of the copper dependent enzyme ferroxidase is found in some cases. After longer exposure the hair looses its color and luster (achromotrichia) and lameness with a characteristic stiff gait may develop. The latter symptoms may be associated with a disturbance of phosphorous metabolism that results in osteoporosis and joint abnormalities (Mills and Davis, 1987). Swayback is a bone or joint disorder seen in young lambs grazing high Mo pastures. Sheep develop a steely wool and exhibit a reduced growth but not the severe diarrhea seen in cattle. Poor appetite is reported in many cases but not all (Lesperance et al., 1986). Suttle (1986b) also concluded that Cu deficiency in lambs lowers resistance to infections such as pneumonia.

All the clinical signs of Mo toxicity may not be due to inhibition of copper metabolism. Severe diarrhea is probably a direct effect of Mo since it is not characteristic of primary Cu

deficiency in cattle. A direct effect of Mo on reproduction has been demonstrated recently. Although Cu deficiency has been implicated in infertility of cattle and sheep, Phillippo et al. (1987a) concluded from a review of the literature that Cu status was not related to fertility. They present evidence that Mo reduces the luteinizing hormone output in cattle which could explain the reduced fertility which they observed. Weanling rats receiving 0, 5, 10, 50 or 100 ppm in drinking water showed no difference in fertility. However, those receiving 10 ppm of Mo or higher had longer estrus cycles, produced fewer and smaller fetuses and reabsorbed more fetuses than those receiving 0 or 5 ppm (S. P. Yang, personal communication). If Mo directly effects luteinizing hormone output, the effect may be the same for ruminants and non-ruminants. Anke et al. (1985) found that one of the symptoms of Mo deficiency in goats is infertility and a high abortion rate.

Evidence indicates that cattle are the most susceptible to Mo toxicity of any species followed by sheep. Horses grazed the teart pastures of Somerset without showing any clinical signs of toxicity. Horses fed Mo did not show evidence of the plasma trithiomolybdate compounds that are the primary cause of the secondary Cu deficiency in ruminants (Strickland et al., 1987). Rabbits (Arrington and Davis, 1954) were shown to be comparatively very tolerant to Mo as are guinea pigs, rats, pigs and chickens (Underwood, 1977). These data were interpreted to mean that processes in the rumen created an environment to enhance the toxicity of Mo by reducing the availability of Cu. More recent data indicates that rumen function may not be the only explanation for species differences because mule deer (Ward and Nagy, 1977) and goats (Anke, 1985) tolerate up to 1000 ppm in the diet or the same as rabbits, rats and chickens. Anke (1985) reports that the toxic level for Mouflon sheep is 300 ppm of Mo.

Water buffalo in China exposed to undetermined levels of Mo in forage developed severe diarrhea and emaciation (Ward unpublished). No clear evidence of Mo toxicity has been reported in humans (Friberg et al., 1975) but it would be expected that tolerance would be much higher than for cattle or sheep as is the case for all non-ruminant species studied.

Calves early in life are non-ruminants and might be expected to be as tolerant as other non-ruminants but in some cases they have developed the same symptoms as cows (diarrhea, joint disorders) on high Mo pastures. However, it has never been determined whether or not these calves had functioning rumen fermentation nor whether the Mo source was feed or milk. Mo is secreted into milk and increased with intake at higher concentrations in feed (Huber et al., 1971). Calves may consume substantial amounts from this source. The toxicity of Mo in milk would be expected to parallel that in water rather in feed. Mo

added to water of young, and possibly ruminant calves, had no effect at 10 ppm and limited effects at 50 ppm (Kincaid, 1980).

Two Holstein bull calves fed either 262 or 411 ppm of Mo, in dry feed, for 129 days developed a mild diarrhea, anemia, lameness, and graying of dark hair and lack of libido. Histological damage to the testes was seen at slaughter (Thomas and Moses, 1951). The significance of these high intakes of Mo to farm conditions may be indicative but not very applicable.

Mo in animal products poses no threat to human consumers. The Colorado State University laboratory (Johnson et al., 1988) has determined with 99 Mo that transfer from the cows diet to milk and meat is respectively 1.7 and 1.0 x 10^{-3} of the daily intake of Mo. The biological half life of Mo in the cow is 2.5 day. Normal milk contains 18 to 120 ppb (Mills and Davis, 1987). The highest levels of Mo reported in milk are 2100 ppb for cows fed 200 ppm of Mo (Vanderveen and Keener, 1964). Human tolerance to Mo is presumably higher than for cattle and it would be unlikely for humans to get a toxic dose of Mo from milk or meat.

DIAGNOSIS OF POTENTIAL MO PROBLEMS

The overt symptoms of severe diarrhea and graying of hair are quite specific to Mo toxicity in cattle. Subclinical expressions such as a lower growth rate, poorer appetite or reduced fertility are difficult to differentiate from many other disorders of cattle (Mills, 1987).

The concentration of Mo in blood plasma, milk or urine is a fairly good indication of intake when Mo intake is high. Plasma or liver concentrations of Cu, although commonly measured, do not provide a reliable estimate of Mo toxicity or the Cu status of the animal. Suttle (1986) concluded that there is no experimental basis for selecting threshold levels for liver copper because there is a range of acceptable levels that coincide with the marginally deficient state. Hair analyses as an indication of mineral status in livestock has not proven very useful (Combs, 1987).

Analyses of forage plants for Mo and Cu can provide some indication of the possible problems but when all the factors are considered that affect an animals response to dietary Mo it is apparent that analytical data of feed cannot reliably predict Mo toxicity. The Cu:Mo ratio in feed has been used to distinguish between safe and hazardous forages as suggested by Miltimore and Mason (1971) and has some indicative value but it suffers from the problems as discussed above. High Mo in soils especially alkaline soils from which plants more readily take up Mo is suggestive of problems but high soil Mo does not necessarily result in high Mo in forage plants.

Ceruloplasmin is the principal carrier protein for Cu in the blood plasma and has been used as an indicator a copper deficiency but is not very closely associated with Cu status or Mo toxicity. Superoxide dismutase is an enzyme in erythrocytes and its activity is a better indicator of the Cu status of chronically deficient lambs (Suttle, 1986b). Determination of this enzyme requires quite sophisticated apparatus.

Clawson (1972) suggests that the best indication of molybdenosis or copper deficiency is the response of animals to copper supplementation.

METABOLISM OF MOLYBDENUM

The interactions of Mo-Cu-S have been known for many years and for the past 40 years research has been underway to elucidate the etiology of molybdenosis and to explain diverse and conflicting response of cattle and sheep to intake of these three elements. Recent research has done much to explain earlier results that often seemed to fit no coherent hypotheses.

The relation of sulfate or organic sulfur compounds to molybdenosis has been particularly difficult to understand because although increased sulfur intake generally increases the Mo effects it sometimes mitigates the effect (Mills and Davis, 1987). Sulfate is a competitor for Mo for carrier sites in the intestine and increased dietary sulfate thus can reduce Mo absorption from the intestinal tract and reduce its uptake from blood by tissues. However, in relation to Mo toxicity the predominant role of dietary sulfur compounds, as sulfate or as sulfur amino acids, is reduction to sulfide under the reducing conditions of anaerobic fermentation in the rumen. Sulfur potentiates the action of Mo as a Cu antagonist in ruminants (Mills and Davis, 1987). Sulfide can combine with Cu to produce an insoluble and unavailable copper sulfide (Suttle, 1986). Sulfide, however, is rapidly absorbed from the rumen (Mills and Davis, 1987) and thus Cu-S is not the major problem and, of course, copper sulfide effects would not explain the relation of Mo to Cu deficiency. Sulfides react with molybdate salts in the reducing medium of the rumen to remove oxygen and produce thiomolybdates. Current theory suggests that the principal relation between Mo and Cu is that thiomolybdates react with Cu to produce soluble but unavailable forms of Cu. Recently, tetrathiomolybdate (TTM) has been identified as the key compound (Allen and Gawthorne, 1986; Kincaid and White, 1988; Mason et al., 1988). The fact that unavailable, TTM bound Cu is found in plasma and liver probably explains many observations where Cu levels in these tissues were not closely related to intakes of Cu, Mo, or S or to the clinical condition of animals. Commonly used analytical methods cannot distinguish between physiologically available and unavailable copper bound in TTM.

Dietary Factors

Suttle (1986b) states that "more progress has been made towards the understanding and control of copper deficiency in sheep and cattle so far this decade than ... in any five year period." In addition to establishing the significance of the TTM molecule, progress has been made in clarifying the effects of dietary components, and factors that limit Cu absorption.

Dietary factors that are clearly related to molybdenosis or Cu deficiency are (1) Cu intake, (2) Cu availability, (3) S intake, (4) Fe intake, and (5) the physical form of the feed.

Copper intake is the primary factor in Mo toxicity because sufficient Cu supplementation can counteract almost all disorders associated with high Mo intakes (Clawson, 1972). It has long been recognized that grazed forage which causes Mo toxicity loses its potency when dried and fed as hay. A six-fold lower Cu absorption has been found in pasture as compared to hay (Suttle, 1986b). Miller et al. (1970) compared the effects of organic Mo, supplied by alfalfa grown on soil fertilized with sodium molybdate, and inorganic Mo added as the salt to the diet of steers. The green-cut alfalfa containing high Mo was more toxic than forage to which Mo salts were added. The authors speculated that differences were due to Cu being less available from the fresh alfalfa rather increased availability of Mo. Cook et al. (1966) concluded that inorganic Mo was more available to rabbits that the Mo in alfalfa but they observed no differences in weight gains or other effects. It has been shown that tall fescue grass but not other grasses resulted in Cu deficiency and responded poorly to supplementation (Stoszek et al., 1986) which indicates differences between plants species. The Cu availability in cereals may be 10 times higher than in forages (Suttle, 1986a).

Continuous feeding (12 times per day) to simulate pasture intake results in a 50% increase in rumen sulfide as compared to once per day feeding, and a 50% decrease in plasma Cu. Practically all of the Cu and Mo found in the rumen is adsorbed to the solid ingesta (Gawthorne et al., 1985). This indicates that rate of movement of digesta to the small intestinal absorption sites will be influenced by feed types and level of intake which influence turnover time in the rumen. Ciliate protozoa in rumen decrease the availability of Cu (Ivan, 1988; Robinson et al., 1987) and protozoal populations generally decrease as concentrates increase in the diet. The results discussed above all suggest that greater Mo toxicity would be expected in grazing animals as compared to feeding the same feeds or equivalent minerals to stall fed animals.

Increased sulfate or total S in the diet generally increases Mo toxicity for both cattle and sheep (Lesperance, 1985; Vanderveen and Keener, 1964) but not in all cases (Goodrich and Tillman, 1966). Increased protein intake has been suggested to reduce the severity of molybdenosis (Bohman et al., 1959). However, increasing protein intake increases the potential for sulfide production for the generation of CuS or TTM. In light of the results described above it may be that the hay supplement supplied a more available Cu source.

The sulfur content of forages ranges from 1 to 6 g/kg of dry weight while cereal grains are lower, in the range 1 g/kg or less. Sulfur in feeds consists of inorganic sulfate-S and sulfur contained in amino acids, which varies with the protein content of the diet. The form of sulfur in the diet makes no difference

as each form can be converted to sulfide in the rumen (Mills and Davis, 1987). Water may also be an important source of sulphates in alkaline soil areas as is the case in the San Joaquin Valley. Data on the S content of feeds is not as readily available as for most mineral elements. Sulfur is required by the rumen microflora for the synthesis of sulfur containing amino acids and some S is recycled in saliva. Sulfur may be deficient in diets containing urea as the principal nitrogen source in which case sodium sulfate is supplemented to the feed. The sulfur requirement is 0.2% (2 g/kg) for lactating cows and lower for other cattle (NRC, 1984).

Copper requirements for cattle have been increased in the past decade from 4.0 to 10 ppm of the dry weight of feed (NRC, 1984). It is not easy to set a Cu requirement because of the many interacting factors which have been described above. In some cases 4 ppm has been adequate for cattle and 10 ppm probably provides a safe margin except in cases where very high levels of Mo are present in the forage. Forages exhibit a tremendous range in Cu content from 1-2 ppm to 25 ppm on a dry weight basis. Low Cu forages are quite common in California and when combined with low availability of Cu in fresh forage results in marginal Cu deficiencies that are more widespread than generally recognized. Cereal grains are lower in Cu than forages ranging from 2-4 ppm. Cattle have a high tolerance for Cu; 115 ppm is suggested by NRC (1984). Sheep have a low tolerance for Cu and care should be taken in using Cu supplements if cattle and sheep are managed on the same ranch.

That iron salts inhibit Cu metabolism has long been recognized but the principal source of Fe for ruminants is soil consumed directly or in feed. It has been assumed that Fe in soils is unavailable. However, it has recently been shown that iron salts from soil are solubilized under the acid conditions of the abomasum (true stomach) to form ferric sulfate which in turn reacts with Cu salts to form the insoluble copper sulfide (Suttle, 1985b).

Soil consumption by grazing animals can be an important source of iron and other mineral intake (Healy, 1971). Intake of soil also can contribute to the Mo intake of animals grazing in areas where soil Mo is high. Cattle grazing alkaline soil also could consume sufficient sulfate to be a factor in reduced Cu availability. Iron salts at 500 ppm reduces copper stores cattle in a manner typical of Mo but had no effect on fertility (Phillippo et al., 1987b). In sheep, 300 to 600 ppm of Fe reduced plasma and liver Cu stores but no overt symptoms of Cu deficiency were reported (Prabowo et al., 1988). Suttle (1986a) suggests that cases of Mo toxicity associated with pastures contaminated with mine wastes are partly due to high intakes of Fe. He also points out that iron oxide is a common and supposedly inert constituent of many mineral mixes for animals.

The Fe requirement for cattle is about 50 ppm (NRC, 1984) and cattle can tolerate about 1000 ppm. The Fe content of feeds varies widely but the major source is probably soil as previously discussed. Some water is also high in Fe but this is not common in the San Joaquin Valley of California.

Several other elements besides Fe have been implicated with the Mo-Cu-S complex. Zn supplements are widely used in livestock feeding and the effect on Cu availability deserves consideration because Zn-Cu interactions have been found in laboratory animals (Mills and Davis, 1987). Fe and Zn behave antagonistically and possibly influence Cu status of animals. High Ca intakes also have been shown to decrease Cu absorption (Mills and Davis, 1987). One study indicates no apparent effect of Se supplementation on the Cu status of dairy cows (Buckley et al., 1986). A recent report shows that monensin (rumensin), a growth promotant widely used in finishing rations for beef cattle, increases the absorption of Se and Zn (Greene et al., 1988). The elements Mn and Cd have been shown to reduce Cu availability to the rat (Nielsen, 1985). The element tungsten (W) is antagonistic to Mo and has been used in the laboratory animals to reduce Mo uptake but no reports have appeared of Mo:W interactions under natural conditions.

Genetic variation is another factor that may explain some of the diverse response to Mo observed in earlier experiments. A difference between breeds of sheep of up to 1.5 times in the ability to absorb Cu has been shown (Suttle, 1986a). None of the breeds studied, however, are common in the U.S. Comparable studies with cattle have shown much less genetic variation than seen in sheep.

A complete assessment of mineral intake requires information about the composition of any mineral mix that is fed as well as an estimate of the quantity consumed. Commercial mixes generally do not state the concentration of the minerals present. Copper is a common constituent but Mo is not. However, one case of Mo toxicity in a dairy herd was reported when magnesium oxide was found to be contaminated with Mo (Ward, 1978).

The manifold interactions of minerals with Cu and Mo are illustrated in Figure 3-1.

TREATMENT OF MOLYBDENOSIS

The treatment of this condition is similar whatever Cu-Mo-S interrelationships may be responsible because the basic metabolic defect in nearly all cases is a lack of sufficient Cu at the tissue level. Treatment may involve a change in feeding practices, such as removal from the pasture responsible, or supplementation with Cu. Daily feeding of Cu as 1 g per head per day of copper sulfate is probably the most effective remedy (Clawson et al., 1972). Copper sulfate can be added at the rate of 1 kg per ton of feed or 0.20 g per liter in water tanks. It should be noted that this level in drinking water is not within the safe limits (0.5 ppm) defined by the National Academy of Sciences (1974). This guideline should not restrict use for therapeutic purposes. Mineral blocks generally contain about .03% Cu. Unrealistic amounts of free-choice mineral supplements generally would need to be consumed to relieve a Cu deficiency.

The most manageable method for supplementing cows or calves on range is subcutaneous injection of Cu compounds which are generally effective for 3 to 4 months. Cu glycinate has been widely used, Cu EDTA causes less abscess problems at the site of injection but other complications have resulted from its use. Copper oxide needles can be administered in capsules. They are retained in the folds of the abomasum where the high pH make Cu slowly available. Cameron et al. (1989) reported that cows dosed with 25 to 50 g of copper oxide had a higher liver stores of Cu. Several experiments have reported the incorporation of Cu and other elements (Co and Se) into a glass matrix which allows long, sustained release of Cu. These boluses are placed in the rumen where they have been reported effective for up to a year (Judson et al., 1985). However, difficulty has been encountered in manufacturing products which provide a consistent and predictable release of Cu. The products have not been approved, to date, for use in this country or Europe.

Rapid and effective response to effective supplementation can be expected in cattle suffering from a definite copper deficiency regardless of whether the deficiency was due to low copper intake or excess molybdenum intake.

Cattle have a rather high tolerance for Cu so the likelihood of Cu toxicity from treatment is not great. Sheep, however, have a low tolerance for Cu. Continuous intakes of 26 to 38 ppm of Cu are suggested to cause toxicity (National Academy of Sciences, 1980). Cu toxicity is rather common in the United Kingdom (Suttle, 1988b). Because of the susceptibility of sheep to Cu toxicity a low level of Mo (1-3 ppm) may be a protection for sheep consuming high Cu diets (Norheim and Froslie, 1985). Mo salts have been added to rations of sheep suspected to be affected by Cu toxicity (Ward unpublished).

DATA FOR MO IN IRRIGATION WATER, ALFALFA AND MILK IN COLORADO

Colorado has been the source of most of the molybdenum produced in the United States. The worlds largest molybdenum mine is located at Climax, a smaller mine at Urad was closed in 1973 and a new large mine was opened at Henderson in 1976. These mines have been closed or on reduced operation during the 1980s. These mines are high in the Rocky Mountains and streams that drain these areas are indicated on the attached Colorado map (Figure 3-2). The drainage from the Climax mine and its tailings ponds is by the Ten Mile Creek to the Dillon Reservoir from which the water flows either down the Blue River or is diverted through Roberts Tunnel into the South Platte southwest of Denver. The Dillon Reservoir was completed in 1962 and prior to this time the Climax mine drainage was entirely through the Blue River. Molybdenosis cases were reported in the early 1950s in cattle grazing meadows irrigated with water from the Blue River. Ranchers who were apparently aware of the difference between green and dry forage subsequently used the land for hay production rather than grazing and had no further problems. Green forage and hay samples ranging from 10 to 57 ppm of Mo on a dry basis and fed to beef cows and calves were analyzed in 1973 (Ward, 1976). This type of hay was presumably fed for some 20 years previously and continued since that time.

In 1971 "The Colorado Molybdenum Project" was funded by the National Science Foundation to define sources and amounts of Mo in Colorado environment transport mechanisms, environmental sinks and the effect on animals and animal food products. Some data obtained from dairy farms should be of interest to California situation and will be briefly reviewed here.

The major agricultural area that is affected by Mo contaminated water is the area north of Denver where irrigation water comes from the South Platte River which obtains water from Ten Mile Creek via Dillon Reservoir and from Clear Creek which drains the area of the Urad mine (Figure 3-2). Water samples in 1972-75 from Ten Mile Creek indicated about 100 ppb of Mo during much of the year. During spring runoff when more water was released from the tailings ponds, levels of 1200 ppb were measured (Runnels et al., 1976). Vlek and Lindsay and his associates (1976) studied Mo levels in soil and uptake by alfalfa in the Brighton area. Ward (1976) studied the relation between irrigation water levels of Mo and alfalfa hay and milk produced by a number of dairy farms in the same area. Irrigation water from ditches on selected farms ranged 0 to 300 ppb. Soils on four farms over four years averaged from 40 to 190 ppb. Alfalfa hay samples from these farms ranged from 2.2 to 9.0 ppm (Vlek and Lindsay, 1976). Alfalfa hay fed to the selected dairy herds, for 35 samples ranged from 2.2 to 5.9 and averaged 3.50 ppm of Mo and 13.5 ppm of Cu. These Mo concentrations are within the range found by Kubota (1977) for legumes in the Western states. There

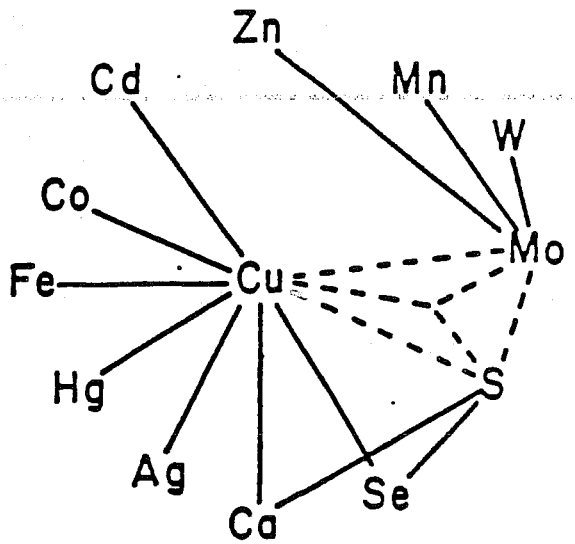

Figure 3-1. Copper

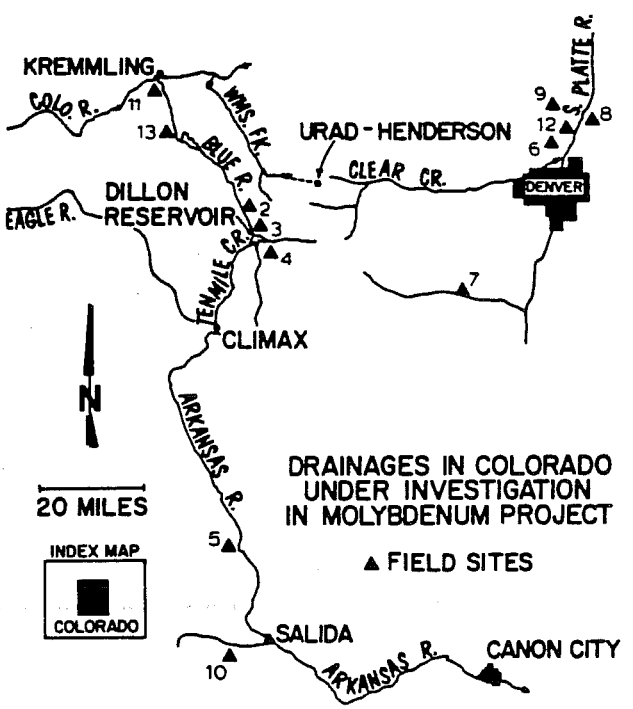

Figure 3-2. River systems an soil sampling sites in central Colorado.

was no detectable Mo in the usual water supplies for the dairy cows. Milk levels averaged 42 ppb which is similar to levels found in other parts of the country. No symptoms of Mo toxicoses were reported at this time or at any time previously or subsequently among dairy cows in Colorado. No toxicity would have been expected with these relatively low intakes of Mo and rather high Cu intakes.

For many years (since 1938 at least) Cu deficiency problems have been suspected in Colorado but not well documented. Jensen et al. (1953) described swayback in sheep in 1953. Forage samples collected in the San Luis Valley in 1966 averaged 4.6 ppm and 3.7 ppm respectively for Cu and Mo and in 1973, 3.5 ppm and 4.2 ppm (Ward and Nagy, 1976). These narrow Cu:Mo ratios have often been suggested to result in deficiency symptoms (Underwood, 1977).

In 1984-85 a survey of the serum Cu status of beef cattle was conducted in western Colorado which again suggests possible marginal Cu deficiency (Tanner et al., 1988). About one-third of 22 herds were judged marginal (less than 0.6 mg Cu/L of plasma)

MO TOXICITY - CU DEFICIENCY IN THE SAN JOAQUIN VALLEY

The history of Mo and Cu research in California has been reviewed by Clawson (1974). A map of defined problem areas is indicated in Figure 3-3. The areas of high Mo and/or low Cu in grasslands or pastures is now well known to cattle and sheep grazers in California and most of them are feeding a Cu supplement of either copper sulfate alone or copper included in a mineral or protein mix. Some use of Cu glycinate injections occurs in cattle operations. Cu deficiency of sheep has been seen but no Cu toxicity problems have been reported although sheep are very sensitive to Cu. Sheep flocks to a large degree are more or less migratory and large numbers are found in the lower valley in the winter where they graze crop residues and alfalfa aftermath. Irrigated pastures are still common in the lower foothills for cattle grazing although this land has increasingly been converted to crop land.

The farm advisor for Kern County (Ralph Phillips) indicates elemental S levels of 0.3% are common in feeds of this county and he feels that 0.4% is a problem level. He has interesting evidence (preliminary) that Mo levels in irrigated pasture and alfalfa appears to have dropped 3-fold over the past 30 years in Kern County where Mo toxicity was first described in 1938. The reason for the decline in Mo levels may be that irrigated agriculture came into this area 30-50 years ago. As Mo is a highly mobile ion under alkaline conditions it is likely native soil conditions showed high Mo levels, however, with continuous irrigation with excellent quality water the soil Mo concentration may have been reduced by leaching. This Mo may now be a portion of the high levels found in the shallow groundwater of the lower San Joaquin Valley. Those areas in the San Joaquin Valley where the streams contain 5 to 10 ppb of Mo are associated with waters high in salt, predominantly sodium sulfate (Westcot, 1989). Cattle have been shown to tolerate 0.4 to 0.7% of sodium sulfate (NAS, 1974) but it would add to the risk provided by Mo.

Veterinary diagnostic services of the University of California-Davis are still collecting some data on blood and liver from farm herds and flocks. Dr. Ben Norman reports that problems have been encountered in a few areas where sulfate fertilizers have been added to the soil. He also sees evidence of an inverse relationship between Se and Cu levels in the blood of cattle and sheep. He finds blood levels of Mo may be indicative of a problem at higher Mo intakes (above 15 ppm) but probably not for levels of Mo generally found in forages in the area. Blood Cu levels have not proved to be of much value in predicting clinical signs of Cu deficiency. Most of the problems in the valley are associated with rather low Mo (2-3 ppm) in feed and medium or low Cu levels.

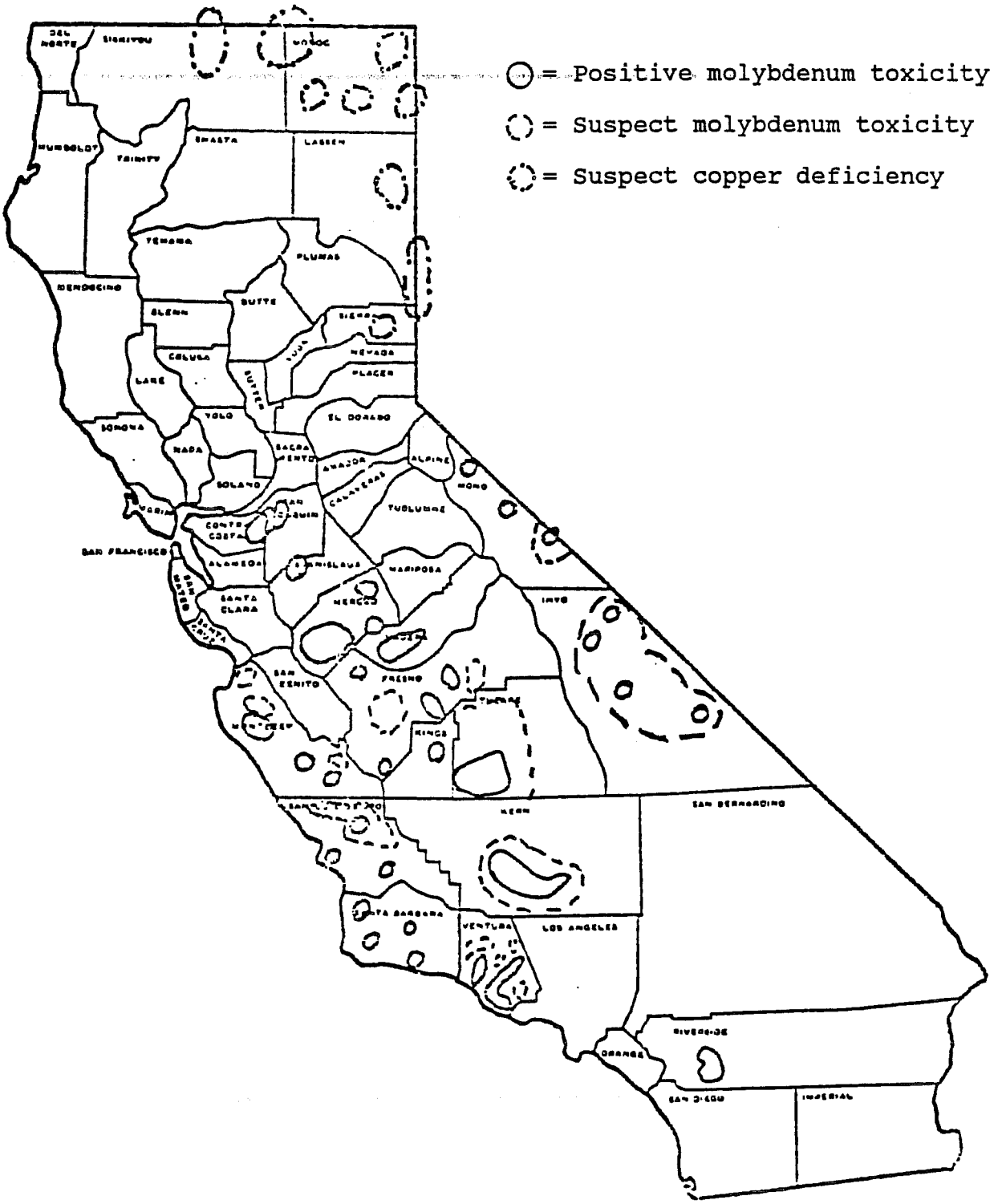

Figure 3-3. Geographic occurrence of molybdenum-copper disturbances of livestock in California (from Clawson, 1974)

SUMMARY OF LITERATURE RELATIVE TO TOXICITY

Considering the many factors described above and the fact that nearly all the evidence is from feed and not water intake, it is very difficult to make positive recommendations for a safe Mo level in drinking water for livestock. It seems clear that ruminants are more susceptible to Mo toxicity because of microbial processes that produce thiomolybdates. The large literature on Mo metabolism in non-ruminants is not included here because of their higher tolerance to Mo. Field observations over the years suggest that Mo toxicity or Cu deficiency occurs at lower Mo intakes in Great Britain and Ireland than in the U.S. If this is true it may be due to the greater number of wet pastures as suggested by Alloway (1977).

What seems clear from observations and research over the years for many areas including California is that Mo toxicity is more likely under grazing situations than those situations where animals are fed stored forage and/or concentrates. This indicates the necessity of considering feeding systems practiced in the San Joaquin Valley especially with a view to determining which feeding system and class of animals is most sensitive. Cattle are considered the species most susceptible to Mo toxicity and it seems evident that grazing cattle will be at the greatest risk because of lower Cu availability and possibly greater intakes of Mo, sulfates, and Fe from forages.

Mo toxicity was reviewed in 1980 (National Academy of Sciences) and Table 3-1 expands on and updates the summary in that publication. Table 3-1 includes only effects that are expressed as clinical signs that affect animal production. The literature contains many interpretations in terms of plasma, liver or other tissue levels of Mo or Cu but these data are not included because of their generally poor correlation with animal response.

The data from early studies of the teart pastures in Somerset indicates that clinical signs were observed at about 14 ppm in pasture with no information available on Cu or S content of forages. In any field study it is difficult to determine the mineral content of the forage or the amount that is actually consumed by animals.

The threshold dose of Mo is from a recent study in England where heifers received 5 ppm Mo added to a barley and straw diet containing 4 ppm of Cu (which is at or below the borderline for recommended levels) from 1 to 3 months of age until breeding age. Estrus was delayed by at least 6 weeks and the conception rate was decreased from 65.8% for controls to 16.7% for Mo treated cattle (Phillippo et al., 1987a). The same research group (Humphries et al., 1983) fed the same ration to growing calves which resulted in reduced growth, bone and joint disorders and

discolored hair. Smith et al. (1975) found abnormal metacarpal and tarsal growth in calves resulting in lameness when they were on pasture containing no more than 6 ppm of Mo (2.2 to 6.2 ppm). No other clinical signs were reported for the calves and the mother cows were reported to be normal. Although effects were seen at about the same concentration of Mo in these two experiments the symptoms were entirely different. Under very similar conditions, as the latter experiments, including high intakes of sulphate in water (about 1%) neither cattle nor calves exhibited any clinical signs or response to Cu supplementation (Cameron et al., 1989).

In a study in western Manitoba (Wittenberg and Devlin, 1987) milking beef cows were fed a barley and silage ration containing 0, 20, and 40 ppm of Mo, 6.0 ppm of Cu and 0.13% sulfur. No clinical symptoms were observed, but milk production declined slightly at the 40 ppm level. No significant difference in the health or weight of calves was observed. Boila (personal communication) also has observed declines in milk production related to higher Mo intake by dairy cows. However, Wittenberg and Devlin (1988) found no effect of 40 ppm of Mo in the feed on milk production of ewes.

In New Hampshire (Vanderveen and Keener, 1964), 5 to 200 ppm of Mo was added to diets of Holstein heifers which contained only 2 ppm of Cu a level considered to be below Cu requirements. Heifers fed up to 50 ppm of Mo developed no clinical symptoms and had normal conception rates. When sulfate at 0.3% was added to the 50 ppm diet, hair was discolored, but no other symptoms were observed over a period of 625 days. All heifers receiving 100 or 200 ppm and 0.3% sulfate developed all the classic symptoms of Mo toxicity.

In Virginia, Huber et al. (1971) added 53 to 300 ppm of Mo to diets containing 6 ppm of Cu. Overt symptoms of Mo toxicity were observed in three cows consuming 173 to 200 ppm of Mo but none in cows receiving 53 to 100 ppm. Sulfate levels were not reported and no reproductive data was provided.

A series of experiments with young beef cattle both under grazing and dry feeding conditions in Nevada were conducted where in most cases Mo was added to the diet at 100 ppm (Table 1). Overt symptoms were always observed at this level. It should be noted that except for Smith et al. (1975) and Cameron et al. (1989) all the data is based upon experiments where salts were added to dry feeds.

A clear conclusion to be drawn from this data is that when 100 ppm of Mo is added to dry feeds that most of the clinical signs of Mo toxicity will be exhibited while in many cases cattle showed slight or no evidence of ill effects below this level. The recent studies in England where reproduction and growth was

severely impaired by 5 ppm of Mo is obviously the threshold level that must be chosen as hazardous (Humphries et al., 1983; Phillippo et al., 1987a).

A threshold level of 5 ppm is clearly indicated but no explanation can be offered for the lack of observed effects when 20, 40, or 73 ppm of Mo was fed to cattle under apparently similar management conditions. A number of dairy herds in Colorado consumed 5 ppm or more in their hay in 1973 and presumably in the years before and since. A few beef cattle herds in the Blue River Valley of Colorado for many years presumably consumed hay containing 10 to 57 ppm of Mo (Ward, 1975). Replacement dairy and beef heifers characteristically consumed only this type of hay without supplements during the breeding season.

If the data available for cattle provides little assurance about safe levels of Mo, the situation with sheep is even more tenuous. In Scotland (Suttle, 1986a) and Ireland (Poole, 1982) grazing areas where fresh forage contains only a few ppm of Mo these Mo levels are suspected of causing Cu deficiency. Ivan and Veira (1985) found that 8.4 ppm of Mo depressed weight gains of lambs but had no other effects. Pitt et al. (1980) found severe bone and joint abnormalities but no other symptoms in lambs grazing pasture sprayed with Mo salts to provide 6 to 12 ppm of Mo. At the other extreme Bingley fed 174 ppm of Mo in dry feed to sheep and observed no clinical symptoms or toxicity (Table 1). The consensus is that sheep are more tolerant of Mo than cattle although there is no direct experimental evidence to support this oft-repeated claim. The data above indicate that the threshold level is about the same for cattle and sheep.

One study each indicates that mule deer (Nagy et al., 1975) and goats (Anke et al., 1985) tolerate at least 1000 ppm. Because horses, poultry and swine apparently tolerate much higher levels than cattle any drinking water guidelines for cattle would be more than adequate for those species.

In most cases grazing animals have been much more sensitive to Mo intake than animals fed dry feed. Mo toxicity has not been reported in California for non-pastured dairy cows or fattening cattle in feedlots where the cattle are fed only dry feed. Copper supplements, it is reported (Don Bath personal communication), are commonly added to diets for dairy cattle in those areas that are suspected of having low Cu and/or high Mo in the hay. Guidelines thus should be focused on grazing cattle and sheep.

GUIDELINES FOR SAFE LEVELS OF MOLYBDENUM IN WATER FOR LIVESTOCK AND POULTRY

No guidelines have been established for Mo in drinking water for animals so it is necessary to develop guidelines based upon the data derived from Mo naturally present in feeds or for most cases where molybdate salts have added to feed. A recommendation of 50 ppb of Mo as the safe level in irrigation water for the west side of the San Joaquin Valley is being considered (Westcot, 1989).

The threshold level to consider for feed is clearly 5 ppm of Mo. No data are available to suggest a safe level lower than 5 ppm. Grazing cattle and sheep should be considered the most susceptible farm animals in the San Joaquin Valley. The problems of defining a safe level of Mo in drinking water for animals becomes difficult if 5 ppm is the threshold level of toxicity, no lower level has been demonstrated to be safe and cattle and sheep in the Valley are frequently consuming forage containing 1 to 3 ppm of Mo, marginal levels of Cu, and water containing sulfate concentrations that may exacerbate Mo effects. The problem is further complicated if any additive effects due to green forage consumption, or high intakes of Fe, Ca or Zn are included in the assessment. The Mo effects on fertility may be independent of Cu, sulfates and Fe intakes but not the pathological changes in bones and joints of cattle and sheep.

A worst case analysis could easily conclude that cattle and sheep should not be exposed to any additional Mo in their diets. This conclusion could be supported by the observations of continuously reported cases of marginal Cu deficiency apparently aggravated by low Mo intakes that are common in the Valley. It is possible that these cases are partially due to Mo in drinking water but there is no data to support this suggestion. Some livestock producers have for many years provided supplemental Cu to their animals to prevent the clinical problems associated with low Cu or high Mo in the feed supply. For worst case analysis it may be appropriate to use the method of the National Academy of Sciences Committee (NAS, 1977) which assigns drinking water 20% of the total exposure to a contaminant and 80% to all other sources. This may be the appropriate method for the conditions of the San Joaquin Valley. The feed of cattle and sheep is known to contain Mo and other elements than exacerbate Mo toxicity.

If 5 ppm is the threshold level and assuming that one-half that level or 2.5 ppm is a safe level then 20% of the 2.5 ppm or 0.5 ppm would be considered a safe intake from water. For the worst case analysis the maximum ratio of water to feed for cattle under heat stress is about 7.5 (Table 2-6). The safe concentration in water then would be 0.5 ppm/7.5 kg of water per kg of feed or 0.067 ppm or 67 ppb. Ray (1989) found a maximum water to feed ratio of 5.0 for feedlot cattle at Yuma, Arizona

under hot, dry conditions similar to or probably more severe than those of the San Joaquin Valley.

Cattle grazing green forage, the most sensitive group, will consume less water per unit of feed than those on dry feed. Sheep consume less water per unit of feed than do cattle. It should be noted that Kincaid (1980) included 10 and 50 ppm in water for young calves consuming diets containing 13 ppm of Cu and found only some decrease in liver Cu at the highest intake.

The conclusion arrived at above that the safe level of Mo in drinking water for livestock is 67 ppb is essentially the same as the recommended lower level for irrigation water and can be rounded off to 50 ppb. A guideline of 50 ppb has been suggested for Mo in irrigation waters of the west side of the San Joaquin Valley. The basis of this recommendation is obtained from the model of Vlek and Lindsay (1977) developed from their studies of Mo in irrigated soils of North-Central Colorado. However, the Vlek and Lindsay model contains the assumption that 10 ppm of Mo in alfalfa is a safe level for cattle. If this assumption were changed to assume that 2.5 ppm, as in this analysis, is the highest acceptable level the guideline for Mo in irrigation would need to be reduced to 12 ppb; a level that might be difficult to achieve.

If the suggested level (50 ppb) is accepted for irrigation water, it would also provide adequate safety for the drinking water supply for grazing cattle and sheep; the most sensitive group of livestock. The worst case analysis indicates a recommended guideline of 50 ppb of Mo in drinking water which should provide safety for cattle and sheep consuming forage containing less than 1.0 ppm of Mo, more than 10 ppm of Cu, and water containing moderate amounts of sulfate. Those farms and ranches where forage Mo exceeds 2 ppm and Cu is less than 10 ppm are routinely supplementing Cu as a precaution against Cu toxicity.

FUTURE STUDIES

A comparison of Mo effects on cattle and sheep when included in water as compared to feed should be conducted. Such a study should include levels at 1, 5, 10, 15, 20, and 40 ppm to delineate a threshold dose and a safe level for both cattle and sheep. At the highest intake, a comparison of Mo in water and feed should be included. Careful attention should be given to Cu and S intake. The threshold dose used in this analysis is based upon the effect of 5 ppm on the fertility of young cattle. This experiment needs to be repeated by other research groups. The effect upon fertility of sheep also needs investigation. A word of caution is that although it is important to know the threshold dose for Mo, the great variability of results in the literature may not indicate a high degree of success for such research efforts.

Research on Cu-Mo interactions is still continuing on a rather large scale especially in Scotland, Australia, and Canada (but not the U.S.) and perhaps a clearer understanding of this complex situation will be possible in the next few years.

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